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A Periodical Devoted to General and Experimental Pathology

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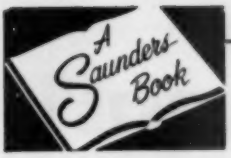

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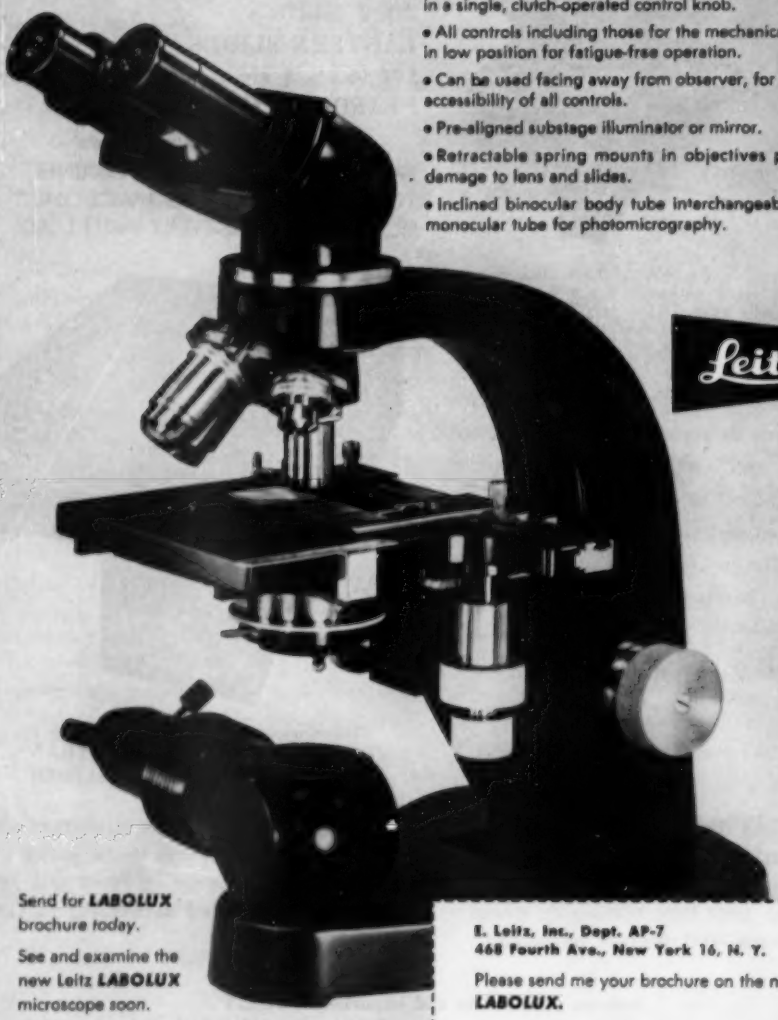

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A.M.A. ARCHIVES OF
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Acquired Toxoplasmosis

Report of a Case with Autopsy Findings, Including a Review of Previously Reported Cases

ANNE DODGE HOOPER, M.D., New Britain, Conn.

A large proportion of adults in many areas have been infected with Toxoplasma gondii as indicated by the relatively high frequency of positive skin reactions to toxoplasmin in different population groups.[1,2] However there are very few instances of acquired toxoplasmosis that have been studied at autopsy. Most infections in older children and adults are subclinical, and most of those persons with clinical manifestations recover. The clinical manifestations are of various types and include one syndrome similar to that seen in infectious mononucleosis (including lymphadenopathy and the presence of atypical lymphocytes in blood smears),[3,4] another syndrome of acute encephalitis,[5] and still a third syndrome resembling the typhus-spotted fever group of diseases in which a maculopapular rash and a high fever occur.[6,7] Most of the autopsied cases have had neurological involvement; in those without neurological involvement death has usually been due to other causes.

Castellani, in 1914, was the first investigator to attribute a death to T. gondii.[8] His case report bears no clinical or pathological resemblance to more recently described cases. In 1940, Pinkerton and Weinman noted the typical lesions of acquired toxoplasmosis in a Peruvian who also had bartonellosis.[9] Because relatively few instances of acquired Toxoplasma infections with autopsy findings have been published, a recently observed case is here presented.

Submitted for publication Feb. 18, 1957.

From the Laboratories of the New Britain General Hospital.

Aided by a Grant from the Research Fund of the Women's Auxiliary, New Britain General Hospital.

Report of Case

A 43-year-old white man was admitted to the New Britain General Hospital in September, 1954, because of hematemesis.

His mother had died of cancer, and his father had died of cirrhosis of the liver. The patient had been in poor health for many years. From 1941 to 1944 he had progressive loss of vision and signs of panhypopituitarism. After unsuccessful radiation therapy, a chromophobe adenoma of the pituitary gland was excised in 1944. There was no further loss of vision, but the loss of body hair which had been observed prior to the operation continued. He was unusually susceptible to infections. He ate a well-balanced diet, smoked very little, and drank very little alcohol. From July to October, 1954, he had a healthy pet dog.

In September, 1954, he developed frequent frontal headaches, mild narcolepsy, and occasional left shoulder pain. A few days later he had cough and coryza. Three days prior to admission he had tarry stools and hematemesis. Physical examination showed him to be afebrile and lethargic. The skin was yellowish-tan, and there was stasis dermatitis of the ankles. He had a moon face, central obesity, scant body hair and beard, and atrophy of the genitalia. The scleras were white. The left pupil reacted slowly to light, and the right reacted normally. Voluntary eye movements were normal, but the eyeballs moved at rest (essentially the same

findings as in 1944). The optic discs were pale. Rales were heard in the left lung base. The heart was not remarkable. The abdomen was soft and tender. No abdominal masses were palpable. Neurological examination was negative.

The erythrocyte count was 3,100,000 per cubic millimeter, the hemoglobin was 10.6 gm. per 100 ml., and the white blood cell count was 5500 per cubic millimeter, with 62% segmented neutrophils, 18% stab forms, 1% eosinophils, 14% lymphocytes, and 5% monocytes. The urine was unremarkable. The total serum bilirubin was 2.2 mg. per 100 ml.; the prothrombin time, 38% of normal; the albumin, 3.5 gm. per 100 ml.; the globulin, 3.7 gm. per 100 ml.; the total cholesterol, 105 mg. per 100 ml.; the cephalin flocculation, 2+; the platelet count, 110,000 per cubic millimeter; the bleeding time, 16 minutes and 45 seconds, and the clotting time 11 minutes 30 seconds. X-rays showed an enlarged heart and spleen. He was given blood transfusions and discharged in 12 days.

The narcolepsy and tarry stools continued. On Dec. 2, 1954, he was readmitted with a two-day history of sore throat and temperature of 102 F, followed by the gradual development of coma over a 12-hour period. The neck was supple, and there were no localized neurological signs. A few ronchi were heard. The heart was enlarged, but there were no murmurs or thrills. The spleen was questionably enlarged. Lumbar puncture revealed normal dynamics, with xanthochromic fluid containing 5650 red blood cells, no white cells, and a protein level of 45 mg. per 100 ml. Cultures of the blood, urine, and spinal fluid were negative. The white blood cell count was 3500 per cubic millimeter, with 59% segmented neutrophils, 14% stab forms, 13% lymphocytes, and 14% monocytes. The sternal bone marrow showed slight erythroid hyperplasia. He suddenly became afebrile and conscious on the day following admission. Cortisone controlled his marked bleeding tendency. He also received penicillin and streptomycin throughout his stay. Eight days after admission he was discharged improved.

He was readmitted on Dec. 19, 1954, because of abdominal distention, dyspnea, and confusion. Examination showed hematomas of the buttocks and antecubital fossae, wheezes, moist rales, hepatomegaly, and splenomegaly. The white blood cell count was 4450 per cubic millimeter. A lumbar puncture showed an initial pressure of 330 mm. of water, and after removal of 8 ml. of clear colorless spinal fluid the final pressure was 260 mm. of water. There were no cells in the spinal fluid. He exhibited a daily spiking fever, with temperature ranging between normal and 105 F. Blood cultures were negative. No malaria parasites could be found on blood smears. He was again given penicillin and streptomycin therapy. At no time during his illness was lymphadenopathy or skin rash noted. Terminally he developed ankle clonus and diminished reflexes on the right. He died six days after admission.

Necropsy Findings

Necropsy was performed eight hours after death. Examination showed a middle-aged white man with central obesity, moon face, scant body hair and beard, slightly enlarged breasts, and atrophic genitalia. The skin was generally bronzed, and over the ankles it was shiny and dark brown. Petechiae were scattered over the body. The scleras were slightly icteric. The lymph nodes were not enlarged. The abdominal cavity contained 300 ml. of clear straw-colored fluid.

Heart: The heart weighed 490 gm. Subendocardial petechiae were present. There was no dilatation. The myocardium was grossly unremarkable.

Lungs: There were numerous fibrous adhesions between the right lung and the diaphragm. The right lung weighed 780 gm., and the left lung weighed 620 gm. The lungs were firm and had collapsed lower lobes. The cut surface was reddish-brown and granular.

Liver and Gallbladder: The liver weighed 1380 gm. It was grayish-brown, with fine nodularity. There was an increased amount of fibrous tissue. A Prussian blue stain was positive for increased quantities of iron. The gallbladder contained stones.

Spleen: The spleen weighed 800 gm. The capsule was thickened and adherent to the diaphragm. The pulp was mushy in some areas and more firm than usual in others. Two accessory spleens were similar to the spleen.

Pancreas, Kidneys, and Pelvic Organs: These organs were not remarkable except for marked atrophy of the testes and prostate.

Endocrine Organs: The thyroid, adrenals, and testes were markedly atrophic. The sella turcica was greatly enlarged; the posterior clinoids were thin, and the anterior clinoids were absent. The pituitary gland was replaced by a fibrotic mass measuring 4×4×2 cm. It contained areas of old hemorrhage and calcification. The optic tracts were buried in this mass. The mammillary bodies could not be identified.

Gastrointestinal System: The mucosa at the esophagogastric junction was ulcerated. A thrombosed, dilated vein was at the base of the ulcer. There were petechiae in the mucosa of the stomach. The stomach contained coffee-ground material, and the terminal ileum and colon contained tarry feces.

The retroperitoneal fat from the region of the pancreas to the pelvic brim was edematous and indurated and contained fibrotic areas. The abdominal lymph nodes were not enlarged and contained no areas of suppuration. The bone marrow was dark red.

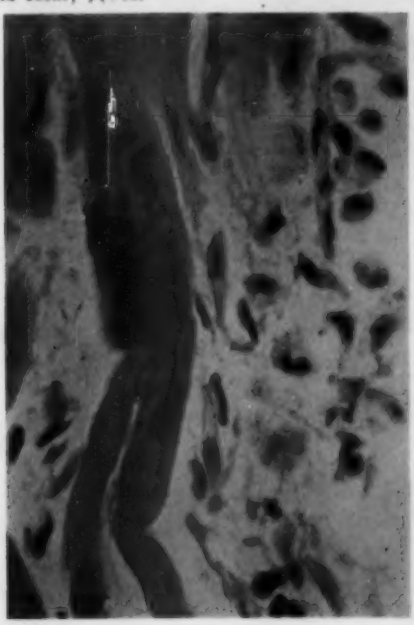
Brain: A well-healed craniotomy incision was present in the right frontal region. There was no epidural, subdural, or subarachnoid hemorrhage. The convolutions of the inferior surface of the right frontal lobe were atrophic. There was no enlargement or displacement of the ventricles. There were no areas of softening, calcification, or hemorrhage.

Microscopic Findings

Heart: The myocardium contained many small foci of necrosis, with infiltration by plasma cells, macrophages, and occasional lymphocytes. Generally well separated from these foci were round to oval collections of small spherical organisms surrounded by a moderately thick wall. These had the typical appearance of the pseudocysts of T. gondii. There were no walls subdividing the pseudocysts. The pseudocysts were both intracellular and extracellular (Fig. 1).

Lungs: The alveolar walls were thickened and contained fibroblasts, macrophages, plasma cells, lymphocytes, and occasional polymorphonuclear leukocytes. Occasional pseudocysts and proliferative forms were seen lying either loose in the alveoli or in the macrophages (Fig. 2).

Fig. 1.—Heart: Pseudocyst in a myocardial fiber. The wall of the pseudocyst is indistinct, and there is very little inflammatory reaction. Hematoxylin and eosin; × 700.



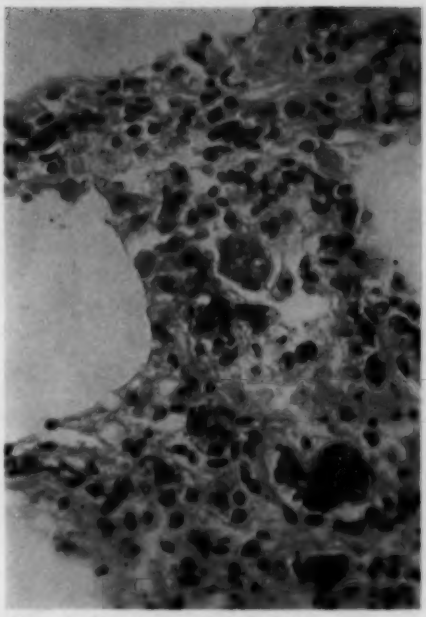


Fig. 2.—Pseudocyst in lung. Hematoxylin and eosin; × 700.

Liver: There was marked fibrosis and dense lymphocytic infiltration of the portal areas and necrosis and minimal fatty infiltration of the hepatic cells. No pseudocysts or proliferative forms were seen.

Spleen: The capsule was covered with an exudate of lymphocytes and macrophages. There was a moderate increase of fibrous tissue throughout the spleen. No pseudocysts or proliferative forms were seen.

Pancreas, Kidneys, and Pelvic Organs: The pancreas and kidneys were not remarkable. The testes and prostate showed extreme atrophy.

Endocrine Organs: The adrenals and thyroid showed marked atrophy. Occasional pseudocysts were present in the colloid of the thyroid (Fig. 3). Almost all of the pituitary gland was replaced by dense fibrous tissue, with dystrophic calcification and heterotopic bone formation in some areas. A few nests of cells similar to those

in the original chromophobe adenoma were present. No normal pituitary tissue could be identified. No pseudocysts or proliferative forms were present.

Gastrointestinal System: Sections through the esophagogastric junction showed chronic ulceration, with many dilated veins. One of these veins communicated through the ulcer into the lumen of the esophagus. One pseudocyst without any inflammatory reaction surrounding it was present in the outer layer of the smooth muscle of the esophagus.

Retroperitoneal Fat: The retroperitoneal fat surrounding the pancreas, adrenals, and kidneys and in the mesocolon and mesentery contained many areas of marked fibroblastic proliferation and diffuse infiltration of plasma cells, macrophages, and lymphocytes. Numerous pseudocysts and proliferative forms were present. There was no fat necrosis (Fig. 4).

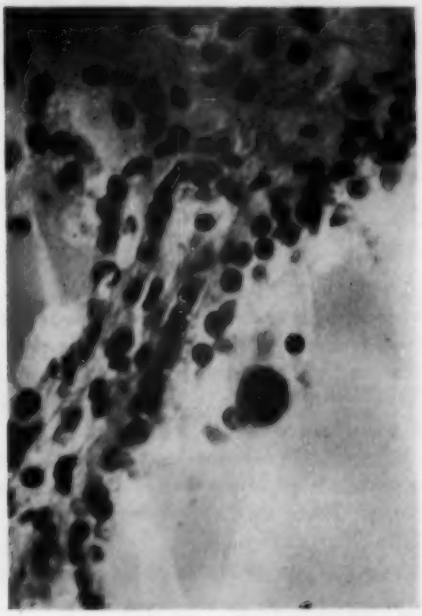


Fig. 3.—Thyroid. Pseudocyst in colloid. Hematoxylin and eosin; × 700.

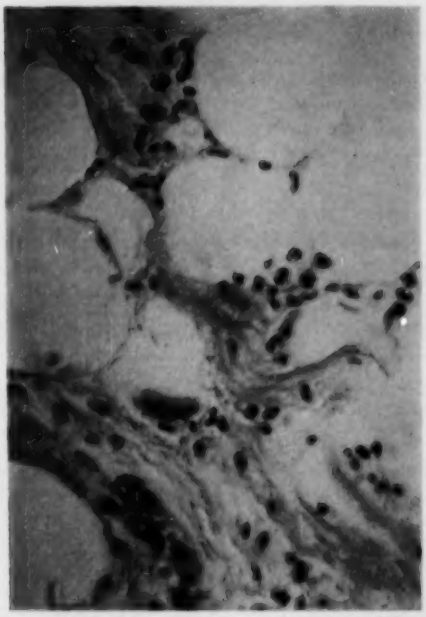
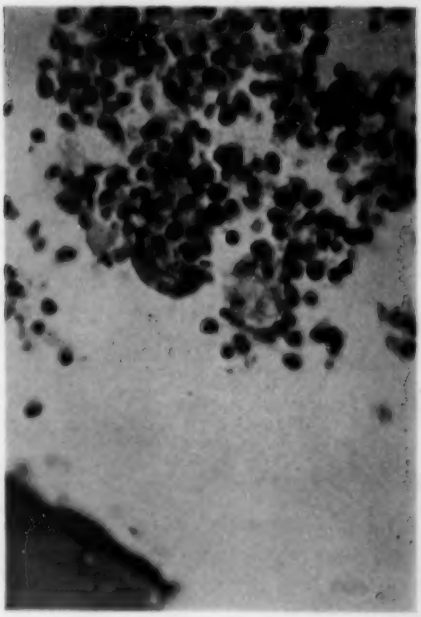


Fig. 4.—Pseudocyst in retroperitoneal fat. There is marked fibrosis and chronic inflammation. A few polymorphonuclear leukocytes are also present. Hematoxylin and eosin; × 375.

Fig. 5.—Pseudocyst in bone marrow. No inflammatory reaction is present. Hematoxylin and eosin; × 500.



Diaphragm: There was loss of striations, necrosis of the muscle fibers, and infiltration by lymphocytes. The nuclei of the sur-

rounding muscle fibers were enlarged. Pseudocysts were seen remote from the areas of inflammation.

Bone Marrow: There was generalized hyperplasia but no inflammatory reaction. Occasional pseudocysts were seen (Fig. 5).

Brain: The meninges contained a slight diffuse mononuclear infiltration. There was no perivascular reaction. Scattered throughout the brain and spinal cord were small microglial nodules with necrotic centers. No proliferative forms and only rare pseudocysts were seen in the nodules. Pseudocysts were scattered throughout the brain and spinal cord, most of them without surrounding cellular reaction (Figs. 6 and 7).

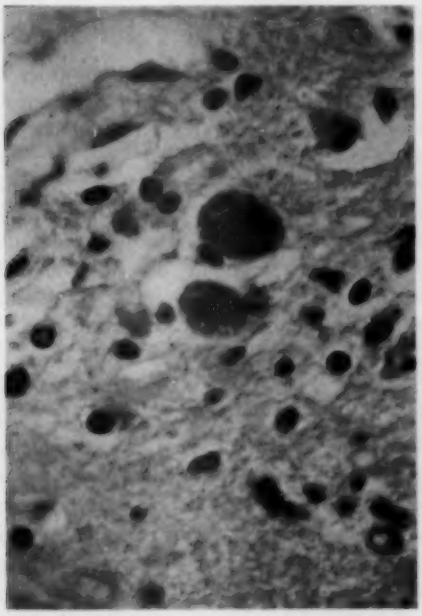


Fig. 6.—Pseudocysts in brain. No inflammatory reaction is present. Hematoxylin and eosin; × 700.

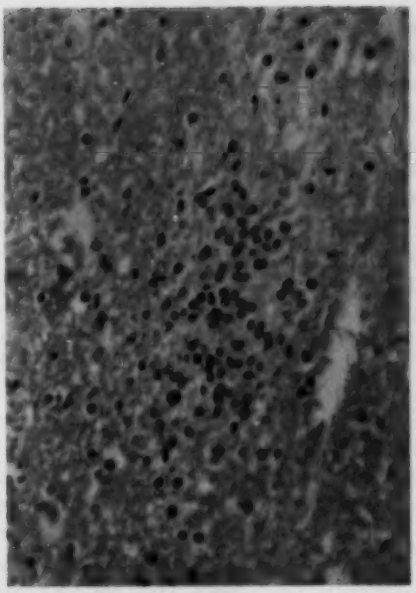
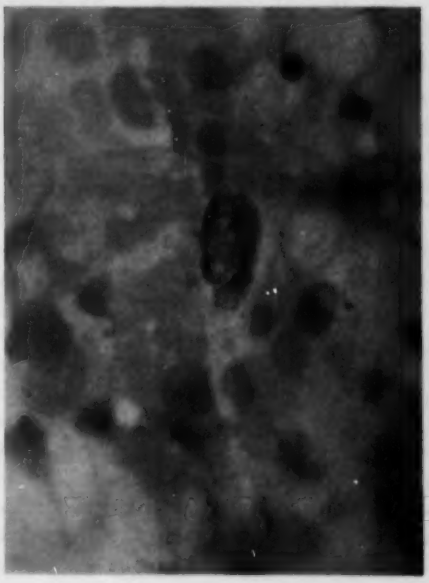


Fig. 7.—Microglial nodule in spinal cord. No pseudocyst or proliferative forms can be identified. Hematoxylin and eosin; × 325.

Comment

The clinical picture and the pathological findings in this case are similar to other cases of toxoplasmosis described in the literature. T. gondii appears in several forms. The free form, seen in body fluid smears, is a lunate nonencapsulated organism measuring 2μ to 4μ in width and 5μ to 7μ in length. It contains a nucleus and glycogen granules and reproduces by binary fission. These forms have been identified in human

Fig. 8.—Pseudocyst in bone marrow smear taken three weeks prior to death. Wright's stain; × 940.



bone marrow smears.[10] No free forms were found in bone marrow smears in this case; however, on reviewing the smears a pseudocyst was found (Fig. 8). In fixed tissue two forms may be found: (1) proliferative form—this form is spherical, occurs in small intracellular groups, and is difficult to identify; chronic inflammatory reactions usually surround these groups; (2) pseudocysts—these are found either intracellularly or extracellularly and consist of ovoid or spherical groups of spherical organisms surrounded by a thick argyrophilic wall. The organisms are closely packed within the pseudocysts, and the cellular outlines are indefinite. The pseudocysts measure 20μ to 100μ in diameter and are unaccompanied by host reaction. Organisms have been found in almost every tissue except erythrocytes.

Sarcocystis, Encephalitozoon, Trypanosoma cruzi, Leishmania, Histoplasma capsulatum, and Cryptococcus (Torula) may have a close resemblance in fixed tissue to Toxoplasma. Biocca feels that Encephalitozoon, Soroplasma, and Toxoplasma should be considered a single genus.[11] Kean and Grocott, in reviewing cases of sarcosporidiosis, have reclassified them into sarcosporidiosis and toxoplasmosis by the morphological characteristics of the pseudocysts in fixed tissue. Those cases in which the pseudocysts were divided into compartments by walls were considered sarcosporidiosis, and those cases containing pseudocysts with a single cavity were considered toxoplasmosis.[12]

To establish the diagnosis beyond all doubt, animal inoculations or serological studies are necessary. Almost any laboratory animal can be infected with Toxoplasma; however, one must be sure that the animals are not spontaneously infected or immune, as naturally occurring infections are widespread. The Sabin-Feldman dye test is the most widely used serological test. The dye test is based on the ability of the serum of a patient who has had toxoplasmosis to prevent the uptake of methylene blue by viable organisms in an alkaline solution. The titer is the dilution of the serum at which half the organisms take up the dye.[13] Recent studies indicate that infection with Sarcocystis,[14-16] Trichomonas vaginalis,[17-18] and Trypanosoma cruzi [18] will cause elevated dye test titers but no positive complement-fixation reactions. The dye test titer rises earlier, goes higher, and falls more slowly than the complement-fixation reaction. In the case presented above, by the time the organisms had been found no fresh tissue or serum was available for study.

Review of Previously Published Cases

The data from 21 published autopsies of acquired toxoplasmosis in older children and adults and the case presented above are summarized in the Table.[19-32] Cases are included which have typical lesions, whether or not the diagnosis was confirmed by serological studies or animal inoculations. Several were originally described as sarcosporidiosis. The examination was limited to the brain and spinal cord in one case.

Pseudocysts were found in the heart in 16 cases. Four of these cases had no inflammation or cellular infiltration, while twelve had focal areas of myocardial necrosis with round-cell infiltration in areas separated from the pseudocysts. Organisms were found in the lungs of three out of four cases with interstitial pneumonia, while they were found in the lungs in only two out of five cases of bronchopneumonia. One had

Adult Toxoplasmosis—Summary of Autopsy Findings of Twenty-One Published Cases and Case Presented Here

Organ	Pseudocysts Present	Inflammatory Lesions Present
Heart	16	16
Lungs	5	13
Liver	2	8
Spleen	3	9
Pancreas	2	0
Thyroid	1	0
Skin	3	3
Lymph nodes	1	7
Skeletal muscle	3	3
Bone marrow	1	0
Fat	1	1
Brain	10	13
Esophagus	1	0
Total cases	20	18

"granulomata" and fibrosis of the lungs with organisms; one had right upper lobe pneumonia without organisms; and one had organizing pneumonia without organisms.

Four cases with portal cirrhosis and two cases with fatty infiltration of the liver had no organisms in the liver, but two cases with focal necrosis of the liver had organisms in the liver. One of two cases with focal necrosis of the spleen had organisms in the spleen; one of three with fibrosis and one of two with malaria pigment in the spleen also contained organisms in the spleen; two enlarged spleens contained no organisms. Organisms were found in the interlobar connective tissue of the pancreas in two cases. No organisms or lesions were found in the adrenals, kidneys, or pelvic organs. Three cases had focal necrosis, round-cell infiltration, and pseudocysts in specimens of skeletal muscle taken from various sites. Although seven cases had enlarged lymph nodes, organisms were found in the nodes in only one case. On biopsy of infected nodes, reticuloendothelial hyperplasia and occasional pseudocysts were found.[33] The skin in three cases showed lesions such as those described in detail by Reich.[34] The rash is nodular, with proliferation of the endothelium of the capillaries and periendothelial histiocytic-lymphocytic granulomata. Some veins show necrosis. Some granulomata involve nerve sheaths. Organisms are seen in the dermis. The case reported in this paper is the only one with pseudocysts in the bone marrow, thyroid, esophagus, and retroperitoneal fat.

In 14 cases brain lesions were found. In a few, there were hemorrhage and gross necrosis, but for the most part the lesions were microscopic. Typically there were small focal areas of necrosis, with infiltration of rare polymorphonuclear leukocytes and occasional mononuclear cells and with marked microglial proliferation. Only rarely were pseudocysts found in or near the nodule. There was very little perivascular infiltration. Occasionally there was a slight mononuclear infiltration of the meninges. In one case there were pseudocysts without inflammatory reaction, and in four cases there were typical inflammatory lesions without pseudocysts.

In cases without neurological involvement death was probably due to liver disease (necrosis and cirrhosis) in four cases, malaria in two cases, drowning in one case, and bacterial peritonitis in one case.

It is interesting to note the relatively large number of cases in which cirrhosis, necrosis, or fatty infiltration of the liver was present (8 out of 22). Not enough data are available to state whether liver disease predisposes to fatal toxoplasmosis or if severe liver disease leading to cirrhosis is an integral part of toxoplasmosis.

Summary

A 43-year-old white man with a long history of panhypopituitarism due to a chromophobe adenoma of the pituitary gland died of toxoplasmosis and portal cirrhosis of the liver. At necropsy pseudocysts of Toxoplasma gondii and chronic inflammatory reactions were found distributed throughout the heart, lungs, thyroid, bone marrow, retroperitoneal fat, skeletal muscle, smooth muscle of the esophagus, brain, and spinal cord. The pituitary showed fibrosis, bone formation, and recent hemorrhage. A few groups of chromophobe adenoma cells were present.

The free form of T. gondii is found in body fluid smears, while the proliferative form and the pseudocyst are found in fixed tissue. No free forms were seen in the bone marrow smears in this case, but proliferative forms and pseudocysts were seen in histologic preparations. A pseudocyst was found in the bone marrow smears.

The complement-fixation reaction and the Sabin-Feldman dye test help establish the diagnosis. The dye test cross reacts with Sarcocystis, Trichomonas vaginalis, and Trypanosoma cruzi.

In a review of previously autopsied cases it was noted that the lesions and organisms are most frequently found in the heart and next most frequently found in the brain.

They are also found fairly often in lymph nodes, lungs, skin, and skeletal muscle. Fatal toxoplasmosis is often associated with severe chronic liver disease.

Dr. Thomas J. Madden, Dr. Paul D. Rosahn, Dr. Theodore Perrin, and Dr. Leon Jacobs helped in the study of this case. The first seven Figures were made by Mr. Howard J. Reynolds. Figure 8 was made by Mr. Alfred Spitzer.

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Enzymatic Changes in Acute Myocardial Ischemic Injury

Glutamic Oxaloacetic Transaminase, Lactic Dehydrogenase, and Succinic Dehydrogenase

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Introduction

Little is known about the early changes that occur in the biochemical composition and histologic structure of well-differentiated parenchymal cells after the sudden onset of severe irreversible injury. However, the later changes are much better documented, and it is common knowledge that irreversible injury to such cells is ultimately followed by necrosis and dissolution of the cell. An inevitable consequence of this phenomenon is the loss to the general circulation of many of those constituents that are concentrated intracellularly. We have recently reported the pattern of the loss of one such intracellular constituent, potassium, from the irreversibly injured, ischemic fibers of experimental myocardial infarcts in dogs.[1] The present paper describes the changes which occur during the first 24 hours after ligation in the activity, and hence concentration, of three intracellular enzymes, glutamic oxaloacetic transaminase (GOT), lactic dehydrogenase (LDH), and succinic dehydrogenase (SDH), from the same infarct.

Methods and Materials

Twenty-one adult mongrel dogs, 16 males and 5 females, were used. These animals were housed in air-conditioned quarters and allowed as much food (Borden's Dog Chow) and water as they wanted.

The animals were divided into an experimental and a control group.

Experimental Group

The experimental group consisted of 13 animals, 9 males and 4 females. Homogeneous myocardial infarcts were produced in the posterior papillary muscle of these animals by a procedure described previously.[2] Briefly, the animals were anesthetized with pentobarbital (Nembutal) and given positive pressure O_2 by tracheal catheter. The left chest was opened under sterile conditions, and the circumflex artery was isolated under the left auricle and ligated at a point approximately 1 cm. from its origin. If the vascular distribution is normal, this procedure results in the production of a large posterolateral infarct, which always includes the posterior papillary muscle. Almost all the fibers in the posterior papillary muscle are involved in the infarct, and this muscle therefore represents an area of homogeneous, irreversible injury which is easy to locate, even though gross findings of infarction may not have had time to develop. Dogs which do not develop cyanosis of the posterolateral aspect of the heart within 15-30 seconds, along with elevation of the ST segments in Leads II and III of the electrocardiogram * are discarded because we have found that the vascular distribution in such animals is atypical and the posterior papillary muscle is not necessarily involved in the infarct.

Animals were killed at 40 minutes and 1, 1½, 3½, 4, 5⅙, 6, 6⅙, 7⅙, 7⅓, 15, 16, 16¾, and 24 hours. The heart was removed from the anesthetized animal and the segment of the left ventricle containing the posterior papillary muscle immediately excised. Samples for chemical analysis were then removed from the upper two-thirds of

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From the Department of Pathology, Northwestern University Medical School, and the Cell Metabolism Laboratory of the Department of Medicine of the Veterans Administration Research Hospital.

* The Sanborn direct-writing electrocardiograph is allowed to run for three or four minutes during and after the ligation.

the muscle. The endocardium was stripped off, as were the attached chordae tendineae, before any tissue samples were taken. Control tissue was obtained from that portion of the anterior superior portion of the interventricular septum which was not involved in the infarct.

The tissue samples were analyzed for water, neutral fat, sodium, GOT, LDH, and SDH by the methods given below.

A complete autopsy was performed on all animals, and sections from normal heart and infarct were studied histologically. No spontaneous diseases were found.

Water; Neutral Fat, and Sodium.—Samples of posterior papillary infarct, marginal infarct, and uninvolved left ventricle were minced in tared weighing bottles and dried at 105 C to constant weight. Neutral fat was extracted and the fat-free wet and dry weight determined by methods described previously.[1] Sodium was determined in a 0.75 N HNO_3 extract of the tissue with a Beckman DU spectrophotometer with flame attachment.

Enzymes.—Samples of infarct from the posterior papillary muscle and control left ventricle adjacent to the samples removed for water and electrolyte analysis, weighing 20-70 mg., were frozen after being weighed in tared glass vials capped with polyethylene stoppers. The tissues were homogenized in a Potter-Elvehjem glass homogenizer. To facilitate homogenization of the muscle tissue, the sample was placed on the inside rim of the homogenizer tube and cut into small pieces with a pair of iris scissors. Homogenates were made in a 1% concentration with M/15 phosphate buffer and aliquots diluted to 0.01%, as indicated below.

Lactic dehydrogenase (LDH) was determined spectrophotometrically,[2,3] using a Beckman DU spectrophotometer. The reaction mixture in final concentrations was as follows: 1.0×10^{-4} M DPNH; 3.5×10^{-3} sodium pyruvate; 20γ heart muscle (0.2 ml. of a 0.01% homogenate), and M/15 phosphate buffer (pH 7.2) to a total volume of 3.0 ml. A water blank was used, as there was no oxidation of the DPNH in the absence of substrate. Changes in optical density at 340mμ were recorded every 5 minutes for 20 minutes. All assays were made at 25 C or the activity corrected to 25 C.

Tissue glutamic oxaloacetic transaminase (GOT) was determined spectrophotometrically by a method analogous to that for serum GOT.[5] The reaction mixture, in final concentrations, was as follows: 3.3×10^{-2} M D,L-aspartic acid; 1.0×10^{-4} M DPNH; 80-100 units of malic dehydrogenase (σ); 6.7×10^{-3} M α-ketoglutaric acid; 20γ heart muscle, and M/15 phosphate buffer (pH 7.2) to a volume of 3.0 ml. The aspartic and α-ketoglutaric acids were neutralized with M K_2CO_3 to pH 7.2. Changes in optical density at 340mμ were recorded every 5 minutes for 20 minutes.

Succinic dehydrogenase (SDH) was determined by a spectrophotometric method similar to that of Slater and Bonner.[6] In the present studies, 1 mg. (0.1 ml. of a 1% homogenate) of heart muscle was used per reaction flask. Optimum concentrations were found to be as follows: 3.7×10^{-2} M potassium succinate; 3.0×10^{-4} M NaCN; 1.0×10^{-3} M $K_3Fe(CN)_6$, and M/15 phosphate buffer (pH 7.2) to 3.0 ml. Changes in optical density at 400mμ were recorded every 2 minutes for 10 minutes.

Calculations: LDH and GOT. One unit was defined as equivalent to a change of 0.001 optical density. Thus, units/min/mg.$=\Delta O.D._{.001/min.}\times1000\times$mg. tissue.

SDH. One unit was defined as equivalent to a change of 1×10^{-5} optical density.† Thus units/min/mg.$=\Delta O.D._{.00001/min.}\times10{,}000\times$mg. tissue.

Control Group

In a series of eight control animals the posterior papillary muscle, the epicardial portion of the left ventricle immediately beneath the posterior papillary muscle, and the anterior superior septum (control L. V.) were all analyzed in order to determine whether these tissues were chemically similar samples of left ventricle. The dogs were anesthetized with intravenous pentobarbital and the hearts removed and tissue samples taken exactly as recorded under the experimental procedure.

Results

The changes in enzyme content which follow the onset of irreversible ischemic injury in the fibers of the posterior papillary muscle are shown in Figures 1, 2, and 3. The enzyme activity of the tissue in units per minute per milligram of fat-free dry tissue is plotted against the number of hours the animal was allowed to survive and a curve drawn that best fits the data.

All three curves have a similar sigmoid shape, with an initial lag period, during which there is little fall in enzyme activity. This is followed by a period of accelerated loss, which lasts until levels of 30%-50% of the average activity of the uninfarcted left ventricle from the experimental animals are reached, 12-15 hours after ligation. Little

† This unit was used in order to make SDH values comparable to LDH and GOT.

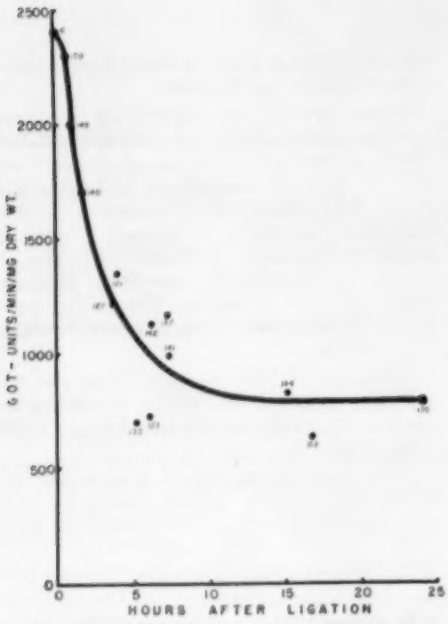

Fig. 1.—Relationship of GOT content of the infarct and the duration of injury. The GOT content is plotted as units per minute per milligram of fat-free dry tissue.

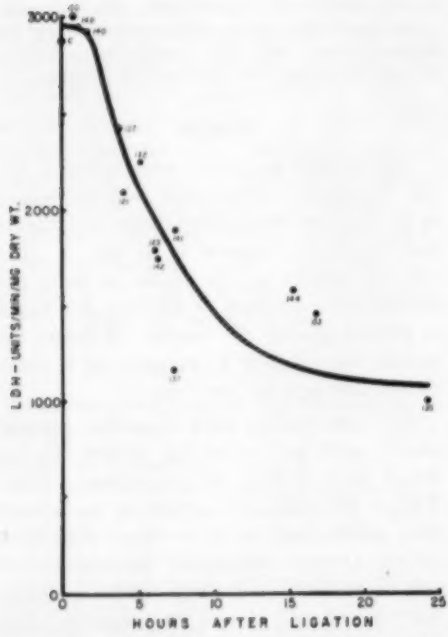

Fig. 2.—Relationship of LDH content of the infarct to time elapsed after ligation. The LDH content is calculated in the same way as the GOT content.

or no further decrease in activity is noted during the next 12 hours.

The duration of the initial lag period is the most variable feature of the enzyme-depletion pattern. GOT, for example, shows an initial lag period of 40-70 minutes (Fig. 1); whereas the initial lag period of LDH (Fig. 2) and of SDH (Fig. 3) is two hours and four to five hours, respectively. However, once these enzymes begin to leave the injured fibers, the rates of loss are approximately the same. The similarity of the slopes of the phase of accelerated loss in the curves shown in Figures 1, 2, and 3 demonstrates this quite clearly.

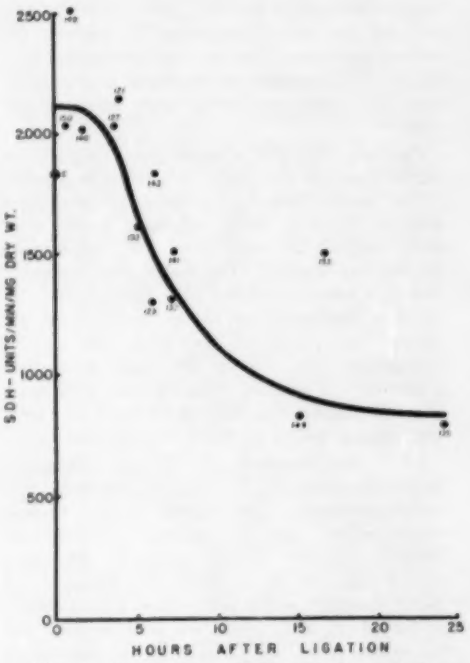

Fig. 3.—Variation in SDH levels in the infarct of the posterior papillary muscle as a function of time elapsed after ligation. SDH is computed in the same way as LDH and GOT.

Chemical results reported in these experiments are calculated on the basis of the dry weight of the tissue. The wet weight is not used because of the speed with which the injured tissue accumulates edema fluid (Fig. 4). This edema fluid dilutes the enzyme-activity levels when they are referred to the wet weight of the tissue and gives values lower than the true activity. The dry-

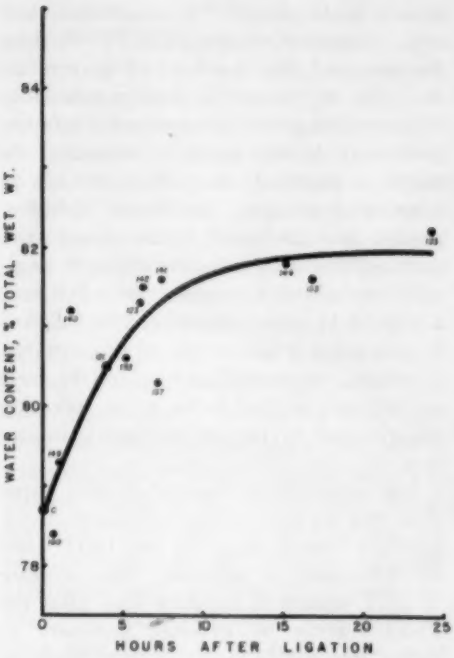

Fig. 4.—Variation in the tissue water content of the infarct with duration of injury. The water content is reported as a percentage of fat-free wet weight of the heart.

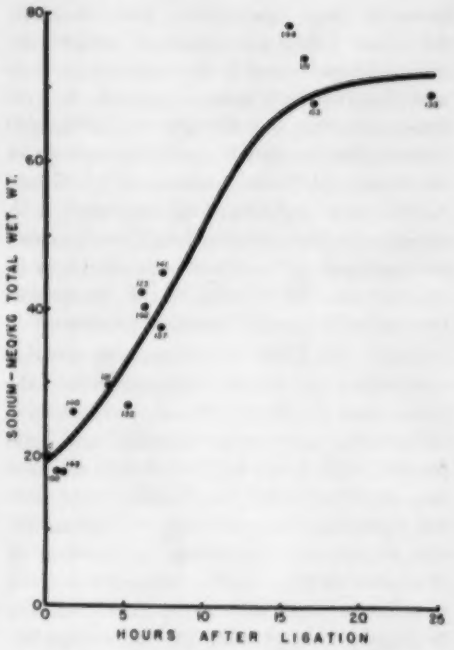

Fig. 5.—Relationship of sodium content of the infarct and the duration of the injury. The sodium content is reported as milliequivalents of Na per 100 gm. of fat-free dry weight.

weight data are therefore considered to represent more accurately the changes occurring within the cell. The sodium content of the posterior papillary muscle infarct increases with the increase in edema fluid (Fig. 5), as might be expected from the high sodium levels of extracellular fluid.

The analyses of GOT, LDH, SDH, Na, and water in the posterior papillary muscle and two other parts of the left ventricle of the control animals are presented in the Table. No significant differences in chemical composition are noted, and the posterior papillary infarct is therefore assumed to be chemically similar to the remainder of the left ventricular myocardium.

Comment

The three enzymes studied in this investigation are all present in mammalian

Comparison of Sodium, Water, and Enzyme Levels of Different Parts of Left Ventricle in Healthy Control Dogs

Constituent	Posterior Papillary Infarct	Left Ventricle Beneath Posterior Papillary Muscle	Anterior Superior Left Ventricle
Water, %	78.57±0.19*	78.52±0.23*	78.84±0.15*
Sodium, mEq/100 gm	20.5 ±0.48	19.4 ±0.64	18.7 ±0.25
GOT, units/min/mg	2364 ±137	2335 ±80.8	2374 ±111.5
LDH, units/min/mg	2864 ±219	2754 ±165.7	2740 ±140.7
SDH, units/min/mg	1635 ±160	2133 ±168	1874 ±148.8

* Standard error of the mean.

All values in this Table are referred to the fat-free dry weight.

The water and sodium analyses were made on duplicate samples from posterior papillary and quadruplicate samples of the other two parts of the left ventricle. The values reported are from five consecutive control animals (Dogs 143, 145, 146, 147, and 148). Data for potassium on these same animals are in a previous paper.[1]

The enzyme data in the Table are the average of eight control animals, including those for which sodium and water values are reported.

heart in large quantities. Two of them, GOT and LDH, are soluble in buffer solutions and are found in the supernatant fluid when heart muscle is homogenized. In vivo these enzymes are thought to be located between the myofibrils in the sarcoplasm of the fiber. SDH, in contrast to GOT and LDH, is a mitochondrial enzyme. It is found with the mitochondrial fraction after homogenization [7] and has been shown to be closely associated with these particulate structures by special staining reactions.[8]

GOT and LDH are known to provide metabolites for aerobic oxidation within the heart muscle fiber. These metabolites—oxaloacetic acid, from aspartic acid, and pyruvic acid, from lactic acid—are oxidized in a stepwise fashion via Kreb's cycle, with the formation of much energy.[9] Catheterization experiments involving the loading of these metabolites in the coronary arterial blood show that both can be readily utilized to supply a large part of the energy required for the continual rhythmic contraction of the heart.[10] A further indication of the importance of GOT in myocardial metabolism is the fact that it is reported to account for approximately 1.6% of the dry weight of the heart.[11]

SDH is an integral part of Kreb's cycle, and the abundance of SDH in all well-differentiated parenchymal cells, including myocardial fibers, indicates its importance in oxidative metabolism. SDH functions by dehydrogenating succinate to fumarate and is also closely associated with the terminal electron transport system (cytochrome-cytochrome oxidase system).[12] The dependence of the heart on aerobic oxidation [10] makes SDH an essential enzyme for maintenance of the integrity of the fiber.

Little or nothing is known about how these enzymes function after the onset of severe ischemia. There is good evidence to indicate that GOT and LDH are not structurally altered by ischemia, because after their release to the general circulation from the dead or dying fibers appreciable amounts of active enzymes can be demonstrated in the serum.[5,13-16] In addition, several groups of investigators [15,17,18] have demonstrated that the level of enzyme activity in the serum is directly related to the size of experimental myocardial infarcts, and hence to the extent of necrosis. As might be expected, other enzymes, such as isomerase, aldolase, and malic dehydrogenase, are also found in the serum after experimental myocardial infarction.[18] Logically, necrosis of myocardial fibers that have a high SDH content should also be followed by increased serum levels of this enzyme. However, increased levels of SDH have not yet been detected in the serum following experimental or human myocardial infarction.

The experiments reported in this paper show that the fibers of the ischemic posterior papillary muscle begin to lose GOT after 40-70 minutes of ischemia. This decrease in GOT content is apparent soon after the 20-30 minutes of ischemia necessary to induce irreversible injury in most fibers of the posterior papillary muscle (Fig. 6) and indicates that there must be defects of sufficient size in the semipermeable mem-

Fig. 6.—Gross photograph of a longitudinal section through the posterior papillary muscle and adjacent ventricular wall of Dog 64, illustrating the fact that 30 minutes of ischemia is sufficient to induce necrosis of many of the fibers of the posterior papillary muscle. The infarct appears pale gray in the photograph and is not as uniform or as extensive as the infarct that results from permanent ligation of the circumflex artery of the dog.[5] This animal was killed three days after the circumflex artery was temporarily ligated for 30 minutes. The circumflex artery was not damaged by this procedure. The animal was allowed to survive for this period of time so that unmistakable signs of infarction would have a chance to develop.

brane of the fiber to allow an enzyme molecule (GOT) with an estimated molecular weight of about 60,000 [7] to leak out. This change in the character if the semipermeable membrane probably occurs within 30-40 minutes after ligation, as no change in enzyme content can be measured by the technique of direct assay used in these experiments until the GOT has diffused from the fiber to the extracellular fluid and thence to the general circulation.

The LDH content of the posterior papillary muscle infarct differs from the GOT content in that it does not begin to decrease until approximately two hours after ligation. As both GOT and LDH are soluble enzymes, it is difficult to explain why they do not begin to leave the fiber simultaneously. Assuming that the damage to the cell membrane increases with time, the only hypothesis now available to explain the longer lag period apparent with LDH in these experiments is the fact that it is an appreciably larger molecule than GOT (135,000 [19] *vs.* 60,000) and therefore requires a longer time interval before it can begin to leak into the extracellular fluid.

The SDH content of the fibers of the posterior papillary muscle infarct does not begin to decrease until approximately four or five hours after ligation. The mitochondrial binding of this enzyme is probably the best explanation for this finding. A two-step loss is postulated, i. e., the SDH must leave the mitochondria before it can leave the fiber, thus causing a delay in the loss of this enzyme from the infarct. Unfortunately, it is not possible to support this hypothesis by relating the time of destruction of the mitochondria in the fibers of the posterior papillary muscle infarct to the SDH curve of loss (Fig. 3) because nothing is known about the timing of the mitochondrial changes in this area. The only detailed study of this subject that we have found in the literature is Wachstein's histochemical study of human myocardial infarcts.[20] He found a qualitative decrease in SDH content in one myocardial infarct two hours old, and in other cases decreases in enzyme content, progressing with time to complete absence of the enzyme from the injured fibers of infarcts three and four days old. The difficulties of timing the onset of human disease plus possible decreases in the SDH content of fibers due to effects of ischemia on fibers supplied by a diseased artery prior to actual acute occlusion of the artery make it impractical to interpret the timing of the SDH loss noted in these data in relation to our chemical findings.

Potassium levels within the posterior papillary muscle infarct approach the extracellular fluid K content 12-15 hours after ligation. The three enzymes studied differ from K in that the 12-15-hour infarct levels are only 30%-50% of the control left ventricle levels. Possibly, stasis within the infarct, due to exudation of protein-rich fluid from injured vessels, slows down the efflux of enzyme from the infarct, or perhaps sufficient enzyme activity is associated with nuclei [21] or mitochondrial [7,22] fragments still present within the infarct at this 12-15-hour interval to result in enzyme activity levels of 30%-50% of normal.

The enzyme changes that are reported in this paper are believed to be characteristic of myocardial fibers injured irreversibly by permanent ischemia. The relationship of these changes to the enzymatic and metabolic changes that occur in reversibly injured myocardial fibers is now under investigation.

Summary

The pattern of loss of glutamic oxaloacetic transaminase (GOT), lactic dehydrogenase (LDH), and succinic dehydrogenase (SDH) from myocardial fibers injured irreversibly by ischemia has been studied in a homogeneous infarct in the posterior papillary muscle of the left ventricle of dogs. After an initial period of little or no decrease in enzyme activity, lasting 40-70 minutes with GOT, 2 hours with LDH, and 4-5 hours with SDH, the tissue levels of all three enzymes rapidly decreased

until levels of 30%-50% of normal were reached 12-15 hours after ligation.

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Effect of Postmortem Autolysis on Certain Histochemical Reactions

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The interest in histochemical reactions as tools in evaluating material obtained at autopsy has greatly increased in recent years. As autopsies are rarely performed immediately after death, and as the postmortem interval is frequently several hours, information regarding the effect of the length of the postmortem interval and the associated postmortem autolysis on histochemical reactions is needed.

This study was undertaken to evaluate the effect of postmortem autolysis on some of the commonly used histochemical reactions: histochemical methods for demonstrating glycogen, succinic dehydrogenase, alkaline phosphatase, and deoxyribonucleic acid (DNA).

Materials and Methods

Twelve healthy adult mongrel dogs were used in this study. The dogs were killed by injecting thiopental (Pentothal) sodium (1.5 gm.) intravenously. The abdomen and thorax were opened immediately after the injection, and the heart, liver, and right kidney were excised. These organs were placed in separate, clean glass jars that could be tightly closed. The organs of six animals were incubated at 37 C, and the organs of six were maintained at 4 C. Two blocks of tissues were taken from each organ at the following postmortem intervals: 0, ½, 1, 2, 4, 6, 12, 15, 18, 24, and 48 hours. In addition, samples of tissue were obtained at 72, 96, 120, and 144 hours from the organs maintained at 4 C. After 48 hours the organs incubated at 37 C were autolyzed to the extent that satisfactory samples could not be obtained. Frozen sections were cut from one block at 30μ and used in demonstrating succinic dehydrogenase. The Seligman-Rutenburg technique,[6] with blue tetrazolium and 30-minute anaerobic incubation (by boiling the substrate), was used. The second block of tissue was fixed in ice-cold (0 C) 90% ethyl alcohol for 24 hours, embedded in paraffin, and sectioned at 6μ. Sections were stained by Gomori's method for alkaline phosphatase[3] by the Feulgen technique for DNA[7] (five minutes' hydrolysis at 60 C was used), by the periodic acid-Schiff (PAS) method, by the diastase-periodic acid-Schiff method for glycogen,[5] by the Brown-Brenn technique for bacteria,[1] and with hematoxylin and eosin.

The succinic dehydrogenase activity was estimated on the basis of the amount of precipitated diformazan, using a scale 0 to 4+ (4+ denoting the maximum activity which was consistently found in the tissue taken immediately after death; 3+, 2+, and 1+ representing 75%, 50%, and 25%, respectively, of the maximum). Using the same arbitrary scale, 0 to 4+, alkaline phosphatase activity and the quantity of glycogen and DNA were estimated.

Results

Morphologic changes were first noted in the cytoplasm and consisted of increased eosinophilia and hyalinization. These changes were evident in the samples taken from four to six hours after incubation at 37 C. Nuclear changes consisting of a decrease in staining with hematoxylin were evident in the 6- to 12-hour period. All of these changes increased with time. The early loss of nuclear staining tended to be focal. On examination of the bacterial stains of the tissue incubated at 37 C, Gram-positive cocci and large Gram-negative rods were found. These were particularly evident beginning at six hours and increasing with time after death. Initially, the bacteria were found at the periphery of the block

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and in and about the larger blood vessels. The focal collections of bacteria corresponded to the focal area where nuclear staining was found to be lost in the hematoxylin and eosin sections.

In the tissue maintained at 4 C there was little evidence of loss of nuclear staining, or of an increase in the number of bacteria even after 144 hours.

Glycogen.—A considerable quantity of glycogen was noted in the liver and myocardium (left ventricle). Little was found in the kidneys. As there was some variation from animal to animal in the amount of glycogen seen in the heart and liver in the samples taken immediately after death, each animal served as its own control; that is, the amount found in the samples taken immediately after death was designated as 4+ and all sections obtained from that animal were compared with it. The amount of glycogen in the heart and liver incubated at 37 C decreased rapidly, so that by 4 hours after death it had largely disappeared and by 48 hours there was very little remaining in the liver (Fig. 1).

The cytoplasm of the cells in sections taken from the tissues immediately after death and digested with diastase to remove glycogen before staining by the PAS method was not colored. However, by 12 hours after death the cytoplasm in the muscle fibers in the heart, epithelium of renal tubules, and cord cells in the liver were a faint pink following diastase digestion. This staining of the cytoplasm by the PAS method following diastase digestion increased some with time after death, but was in no instance as marked as has been seen in experimentally produced myocardial infarcts.[4,10] The PAS-positive material is not glycogen, as prolonged digestion with diastase does not remove it. It presumably represents some breakdown product associated with necrosis.

Succinic Dehydrogenase.—An appreciable amount of succinic dehydrogenase was found in the cord cells of the liver, renal tubules, and myocardium in the samples of tissue taken immediately after death. There was a considerable decrease in succinic dehydrogenase activity in the tissue

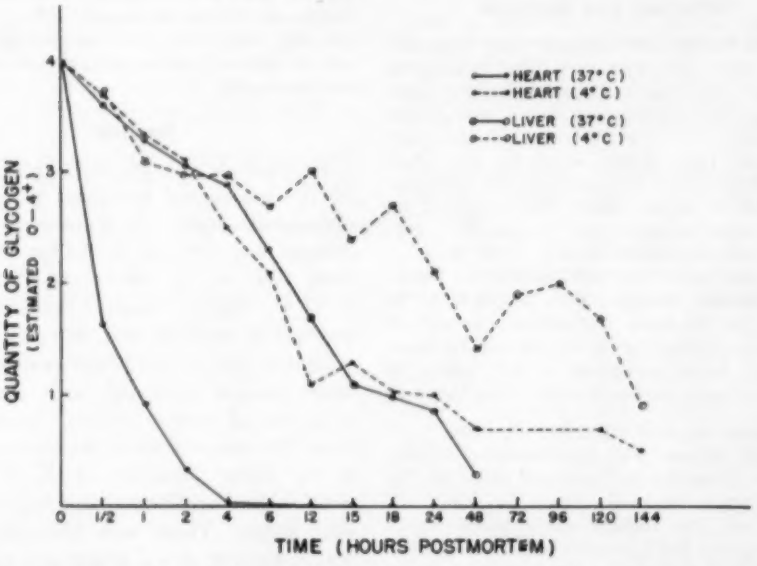


Fig. 1.—The quantity of glycogen in the heart and liver incubated at 37 C and at 4 C is shown at different times after death. Each point represents the mean of six determinations. Note the rapid decrease in glycogen in the heart incubated at 37 C.

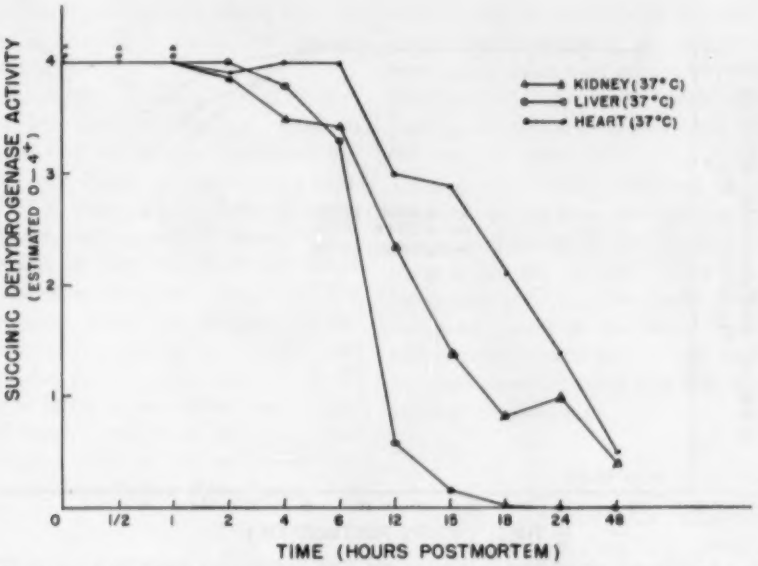
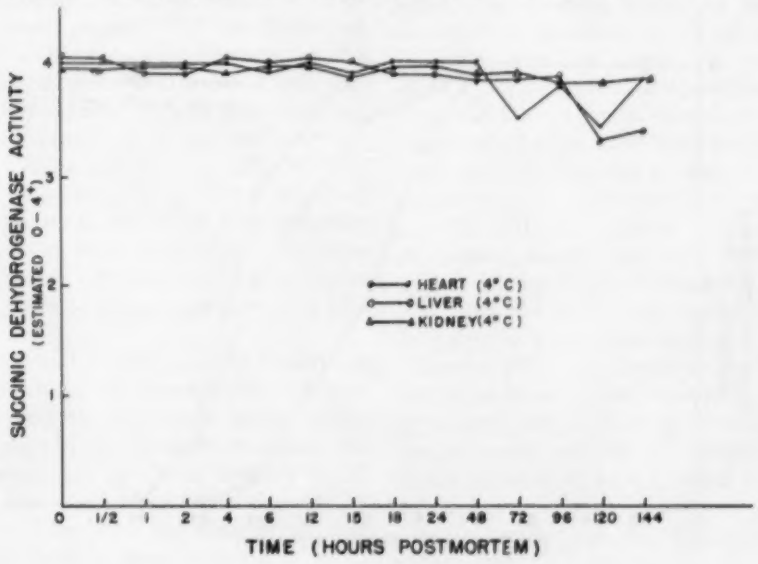


Fig. 2.—Succinic dehydrogenase activity in the heart, liver, and kidney incubated at 37 C. Each point on the graph represents the mean of six determinations.

incubated at 37 C 12 hours after death. The decrease was particularly evident in the liver, and less so in the kidney and heart (Fig. 2). At 48 hours there was very little succinic dehydrogenase activity in any of the organs. The tissue maintained at 4 C showed no significant change even 144 hours after death (Fig. 3).

Fig. 3.—Succinic dehydrogenase activity in the heart, liver, and kidney of tissue maintained at 4 C. Each point represents the mean of six determinations. Note the stability of the reaction as compared with that in tissue incubated at 37 C (Fig. 2).



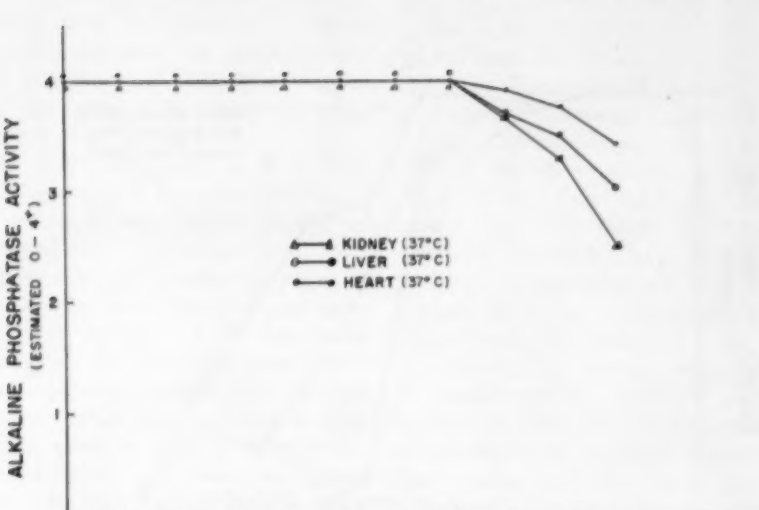
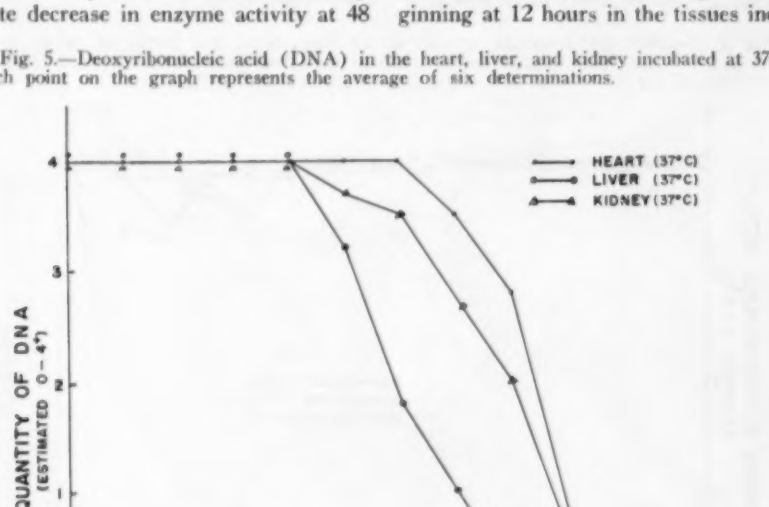


Fig. 4.—Alkaline phosphatase activity in the liver, kidney, and heart incubated at 37 C. Each point on the graph represents the mean of six determinations.

Alkaline Phosphatase.—Alkaline phosphatase activity was noted in the cord cell of the liver, renal tubules, and small blood vessels of the myocardium. There was only moderate decrease in enzyme activity at 48 hours in the tissues incubated at 37 C and no appreciable decrease in enzyme activity in the tissues maintained at 4 C (Fig. 4). However, diffusion was very severe, beginning at 12 hours in the tissues incubated

Fig. 5.—Deoxyribonucleic acid (DNA) in the heart, liver, and kidney incubated at 37 C. Each point on the graph represents the average of six determinations.



at 37 C. There was only a slight increase in diffusion at 144 hours in the tissues maintained at 4 C.

Deoxyribonucleic Acid (DNA).—Deoxyribonucleic acid (DNA) was found in all the nuclei of the tissues examined immediately after death. In the tissues incubated at 37 C there was a sharp decrease in DNA between 6 and 12 hours and a complete loss of DNA in all of the tissues thus incubated at 48 hours after death. On the other hand, there was no apparent decrease in DNA staining even after 144 hours in the tissues maintained at 4 C. The loss of DNA in the earlier time periods (6 to 12 hours) was focal, and this focal loss correlated with focal collections of bacteria as seen in the Brown-Brenn stain (Fig. 5).

Comment

At least four factors are operating in the postmortem period that are important in determining the effect on the histochemical reactions that have been studied: the time interval, the temperature at which the tissue is maintained, the amount of bacterial growth, and the type of tissue. The importance of time and temperature during the postmortem interval is evident in all of the reactions studied. The correlation of growth of bacteria with decrease in the histochemical reactions for DNA and succinic dehydrogenase is particularly evident. The more rapid loss of succinic dehydrogenase, alkaline phosphatase, and DNA by the liver and kidney as opposed to the heart indicates a tissue difference in postmortem breakdown of these components. This may be due to the relatively high concentration of proteolytic enzymes in the liver and kidney.[6]

Mowrey and Bangle[6] observed relatively little histochemically demonstrated glycogen in the hearts of non diabetic human adults at autopsy. In the 63 cases examined, the postmortem interval varied from 2 to 22 hours. The rapid postmortem decrease of glycogen in the liver and heart of the dog tissue, particularly those incubated at 37 C, suggest that the paucity of glycogen in the human heart obtained at autopsy several hours after death may be due to postmortem breakdown of glycogen, rather than to a scarcity of glycogen in the myocardium at the time of death.

Gössner,[3] working with rats, described a decrease in alkaline phosphatase activity, and also diffusion of the reaction for this enzyme in the kidneys after 12 hours' incubation at 37 C. This is essentially what has been found in the dog. The loss of succinic dehydrogenase activity in the dog was much more striking than was noted with alkaline phosphatase.

Summary

The effect of postmortem autolysis on the histochemical reactions for glycogen and DNA content, succinic dehydrogenase, and alkaline phosphatase activity of the liver, kidney, and myocardium (left ventricle) has been studied using dogs. Organs of six animals were incubated at 37 C. Samples were taken at 0, ½, 1, 2, 4, 6, 12, 15, 18, 24, and 48 hours after death. The organs of six animals were maintained at 4 C, and samples of tissues were taken up to 144 hours. The glycogen content of the heart and the liver decreases rapidly after death, especially when the tissues are incubated at 37 C. The succinic dehydrogenase activity and DNA content were markedly decreased by 12 hours after death in the tissues incubated at 37 C, although there was little change noted in the tissues incubated at 4 C, even after 144 hours. The alkaline phosphatase activity was only slightly decreased in the tissues incubated at 37 C even after 48 hours, and was not at all decreased in the tissues incubated at 4 C even after 144 hours. Considerable diffusion was noted in the tissues incubated at 37 C, particularly after 12 hours' incubation. There was also some evidence of diffusion in the tissues maintained at 4 C after 24 to 48 hours.

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Experimental Aberrant Lipogenesis

III. Tissue Factor

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To study the cell response in experimental aberrant lipogenesis, the same techniques were employed as described in the previous reports on the serum factor [1] and the substrate factor [2] except that variations were introduced in the tissue. Thus, a substrate factor, usually oleic acid or sodium oleate, was injected (1) into the cornea or other tissue of living rabbits; (2) into excised buttons of cornea or other tissues, which were then incubated in serum, or (3) directly into the incubation media. At varying intervals the tissue was removed, sectioned in the frozen state, and stained by the Sudan IV and other methods, as directed to the specific question under investigation.

The major interests in this study centered about, first, the processes within the cell that culminated in the formation of fat and, second, the comparison of lipogenic activity in different tissues of the body. For most of the experiments rabbit tissue was used, and the cornea was studied most extensively. The results will, therefore, be first described for the cornea and then for the other tissues.

A. Cornea

The experiments, previously described, dealing with the serum factor and substrate factor provided considerable information on the corneal response. Accordingly, the sections of these experiments were analyzed from the tissue point of view and supplemented by experiments directed toward the following aspects: type of cell capable of lipogenesis, variation with species, chronology of events, effect of pH and specific buffers, temperature of incubation, enzyme inhibition in the tissue, direct testing for lipase, aerobic and anaerobic incubation, and exposure of the tissue to miscellaneous conditions prior to incubation, as well as during incubation. Since lipogenesis necessitates survival of the cells, some studies were directed toward preservation of cells in different milieu. Also studied was the effect on the lipogenesis of mechanical trauma, chemical injury, and radiation. These results will be presented seriatim. Several hundred separate experiments were conducted for this part of the investigation.

Cell Type.—All the cells of the cornea (epithelium, stroma, and endothelium) were capable of showing lipogenesis, and the threshold was approximately the same for all; i. e., when abundant in one type of cell, it was abundant in the other types also. With substrate dissolved in the medium, lipogenesis was present uniformly in all cells. The basal cells of the epithelium, however, showed lipogenesis earlier and somewhat more intensely than did the superficial epithelial cells, and, for some reason which is not apparent, the stromal cells adjacent to the cut edges of the corneal buttons often showed more intense lipogenesis than did the stromal cells elsewhere.*

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Howe Laboratory of Ophthalmology, Harvard Medical School, and Massachusetts Eye and Ear Infirmary.

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* These cells adjacent to the edge were also larger after the incubation and contained more basophilic material than did the stromal cells elsewhere.

When the oleate was injected directly into the cornea, lipogenesis was most marked in the surviving adjacent cells. Occasionally surface cells (epithelium or endothelium) showed abundant lipogenesis when the stromal cells had disappeared entirely. Apparently the stromal cells were somewhat more susceptible to the toxic effects of oleate than were the surface cells.

No difference was found in lipogenesis of axial and peripheral portions of the cornea. Blood vessels, which were often fortuitously present in the latter, showed somewhat less lipogenesis and somewhat greater resistance to the toxic effects of oleates than did the adjacent stromal cells.

Effect of Hydrogen Ion Concentration on Lipogenesis

	McIlvain Buffer						
pH (initial and final)	4.09–4.1	4.5–4.55	4.95–4.95	5.6–5.7	6.09–6.1	6.5–6.6	7.0–6.7
Lipogenesis	0	0	0	±	+++	+++	+++

	Michaelis Buffer							
pH (initial and final)	8.1–7.85	8.4–8.1	8.07–8.2	8.52–8.69	9.0–9.1	9.59–9.5	10.1–10.05	10.0–10.4
Lipogenesis	+++	+	+	0	0	0	0	0

Epithelium, when separated from the stroma, showed full lipogenesis, as did the stroma when separated from the epithelium (and endothelium). Thus the phenomenon did not depend on the intactness of the whole cornea.

The fat globules developed exclusively in the cytoplasm, never in the nuclei. Under favorable conditions it appeared that the globules formed first immediately about the nucleus. Attempts to correlate the site of fat formation with mitochondria were inconclusive.

Lipase activity of the corneal cells was studied histochemically by Gomori's technique [8] and chemically by measuring titratable acid liberated by incubating polysorbate 80 U. S. P. (Tween 80) with ground-up cornea. Pancreas was used as a control. No evidence of lipase activity was found histochemically in the normal cornea or in corneas which had been injected with neutral fat 3 and 30 days previously. Nor was lipase activity found by chemical means.

Species.—Lipogenesis occurred equally in fresh corneas from human, beef, cat, rat, guinea pig, and rabbit sources.

Chronology of Events.—Injection of oleic acid in corneas which were then incubated, or direct incubation of corneal buttons in serum-oleate media, resulted in the appearance of fat first at six hours. The quantity of fat continued to increase for five days, with no further detectable increase up through seven days. Initially the fat formed in small globules at the microscopic limit of resolution of the high-dry magnification. These globules increased in size and eventually coalesced, but, in contrast to adipose tissue of depot fat, they tended to remain as discrete globules within the cytoplasm.

Hydrogen Ion Concentration.—Corneal buttons were incubated in serum-oleate, the hydroxyl concentration of which was controlled by the use of standard McIlvain and Michaelis buffers. The pH of the medium was determined at the beginning and at the end of the period of incubation. The results are presented in the accompanying Table. No lipogenesis occurred with hydroxyl concentrations less than pH 5 or more than pH 8.5, but abundant, and apparently equivalent, lipogenesis occurred in the pH range of 6-8.

Temperature of Incubation.—Corneal buttons incubated for 24 hours at room temperature (20-24 C) yielded moderate lipogenesis, while buttons incubated for the same length of time at 37 C yielded abundant lipogenesis. At the extremes of temperature, 4-5 and 55-60 C, no lipogenesis occurred in otherwise comparable experiments.

Enzyme Inhibition.—Heating corneal buttons to 60 C for periods of five minutes to one hour, prior to incubation, completely abolished the capacity for lipogenesis on subsequent incubation. Potassium cyanide added to the incubation medium diminished lipogenesis in concentrations of 0.0025 M and abolished it completely in concentrations of 0.02 M. Dinitrophenol added to the medium diminished lipogenesis in concentrations of 0.00066 M, but did not abolish it completely even in concentrations of 0.0025 M.

Incubation in an atmosphere of nitrogen for 24 hours diminished but did not prevent the lipogenesis.

Survival of Lipogenesis as Function of Storage.—The capacity for lipogenesis was preserved fully by storage of the corneas in moist chambers in the refrigerator at 2-5 C for five days. It was preserved but attenuated in 5-9 days and was abolished completely in 11 days or longer.

Survival of Lipogenesis with Miscellaneous Treatment of the Corneal Buttons.—Some idea of the ruggedness of the lipogenetic process was obtained by subjecting the corneal buttons to a variety of traumata prior to incubation and during incubation.

Prior soaking of the buttons in distilled water for times as short as one-half hour before incubation resulted in abolishing lipogenesis completely. On the other hand, no reduction in lipogenesis was found on soaking the buttons in isotonic saline solution for 18 hours (at either room temperature or 2-5 C) or with soaking in Tyrode's solution for as long as 4 days. In a single experiment, corneal buttons were incubated in chicken-embryo extract for two weeks without any reduction in lipogenesis.

Corneas irradiated by means of strontium beta emission showed no reduced capacity for lipogenesis after receiving 15,000 rep. This was true whether the corneas were incubated immediately after the radiation or one month later (in which case, the radiation was applied in vivo).

Mechanical traumata, on the other hand, had markedly deleterious effects on corneal cells, and thereby on corneal lipogenesis. This effect was tested by exerting pressure on the buttons prior to incubation. Slight pressure from forceps or from a knife blade was sufficient to result in complete disappearance of the injured cells on subsequent incubation.

Comment.—The foregoing observations on the cornea are interpreted as follows: The phenomenon of aberrant lipogenesis is an intracellular property common to all native cells of the cornea of diverse animal species. It appears to result from an enzymatic process with a wide pH optimum about neutrality. Its inhibition by cyanide and dinitrophenol points to metal-dependent (presumably iron or copper) and phosphorylating energetics. From a practical point of view, it is noteworthy that the property of lipogenesis is well retained with storage of the eye at 2-5 C for five days † or with storage of the buttons in Tyrode's solution for at least four days. Immersion in distilled water, however, is not tolerated for more than a matter of minutes.

The lipogenic property, or the cells containing it, are resistant to heavy doses of ionizing radiation (15,000 rep) but extraordinarily sensitive to mechanical traumata.

† It is of some interest that Duane[4] and de-Roetth[5] reported that the metabolism of stored cornea remains normal for approximately the same length of time.

The function of the rest of the paper will be to report the results of analogous tests done on other tissues. Of these, the liver was studied most extensively and will therefore be described in some detail.

B. Noncorneal Tissue

1. *Liver.*—Liver was selected for a detailed study of experimental lipogenesis in part because it was composed of parenchymatous tissue quite distinct from the predominantly fibrous tissue of the cornea and in part because of its central role in normal lipid metabolism. It was felt that if two such diverse tissues showed similar properties of lipogenesis, the phenomenon might be expected to be a widespread, if not universal, phenomenon.

The liver cells differ from the corneal cells in several pertinent respects. In the first place, some of the cells normally contain fat that can be stained with Sudan. This is especially prominent in the livers of animals which have not been fed for 12 hours or longer. This fat we shall call indigenous fat, as distinct from the experimentally induced, or so-called lipogenic, fat. Indigenous fat offered no great problem in differentiation from lipogenic fat inasmuch as it had a different distribution (as will be described), and it was fully metabolized and disappeared in the process of incubation.

Second, liver cells differ from those of the cornea in containing an abundance of glycogen, thereby permitting a histochemical study of the relationship of glycogenolysis to lipogenesis, particularly the separate participation of these two in such processes as necrosis and starvation.

The technique for studying lipogenesis in the liver was kept as similar as possible to that of the cornea. Either oleates and other substances were injected into the liver of living animals, or else liver pieces, measuring several millimeters on a side, were incubated in the test tube with serum-oleate or plain serum.

The lipogenesis in the living liver was similar to that in the cornea when oleates were injected insofar as vigorous fat formation occurred in the adjacent surviving cells. However, oleates were not an essential prerequisite, as any necrotizing agent induced the same lipogenesis. This is perhaps not surprising in view of the fact that necrotic liver had previously been shown to contain native lipids (presumably oleates), which provided adequate oleate-simulating substrate. Similarly, and presumably for the same reason, liver buttons incubated in serum were capable of active lipogenesis, even in the absence of oleates. No fat formation occurred with incubation in 0.9% sodium chloride, but some fat was inconstantly formed with Tyrode's solution (possibly attributable to the serum in the liver buttons). A further striking and constant difference in the lipogenesis of the liver buttons as compared with that of the corneal buttons was the fact that fat formation occurred only in the marginal cells of the former, that is, in the few cell layers adjacent to the medium. Often, but not invariably, the most marginal one- or two-cell layers were necrotic and the lipogenesis occurred in the adjacent surviving cells. This applied to both the capsular and the noncapsular (cut) surfaces.

The reason for the limitation of the lipogenesis to the marginal cells was not apparent. Obvious possibilities are limitation in diffusion of essential serum factors or of oxygen. The former seems unlikely in view of experiments in which serum was injected into the liver button prior to incubation. This did not succeed in inducing lipogenesis within the substance of the button. The possibility that limitation of oxygen diffusion was the reason for the marginal localization has previously been suggested to explain an analogous observa-

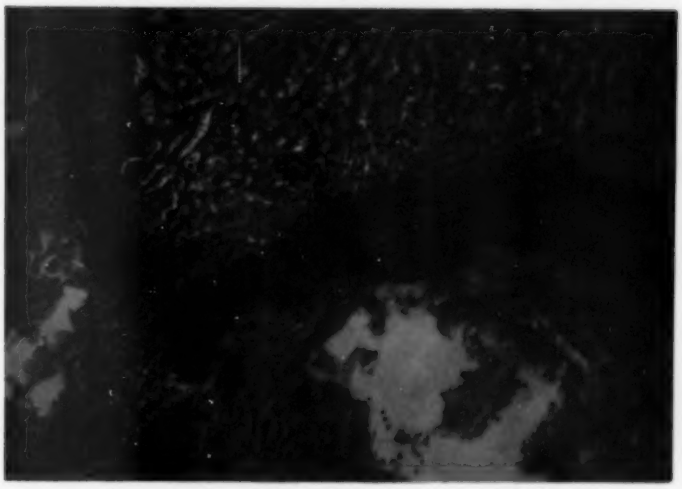

Fig. 1.—Zone of sudanophilia in rabbit liver adjacent to site of necrosis induced by injection of hydrolyzed rabbit fat in vivo six hours previously The fat was situated within cells, and these appeared to be normal. Little or no fat was present in the necrotic area itself. Similar results were obtained by any necrotizing agent (e.g., injecting boiling water or freezing liver).

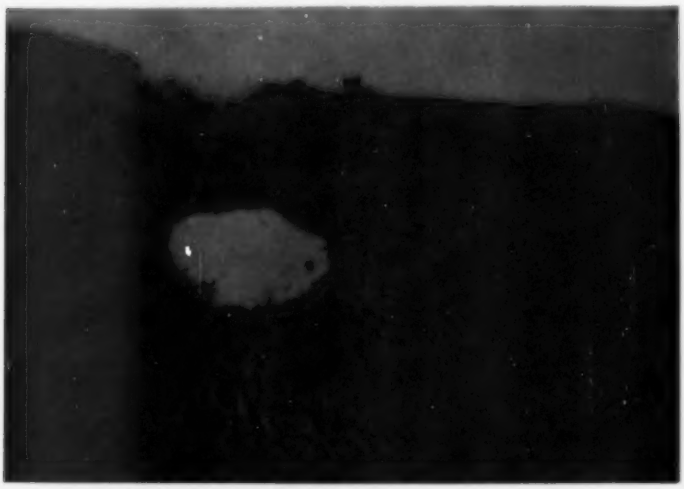

Fig. 2.—Liver button which had been incubated in serum for 24 hours. Fat formation occurs characteristically in a marginal zone and to a less extent about the marginal veins. There may or may not be a marginal zone of necrosis. Unlike the corneal experiments, the serum does not need to be fortified with oleates, as liver can use its own fatty acids for synthesis of neutral fat.

tion in the case of in vitro synthesis of glycogen by liver.[6] This possibility is further substantiated by the diminution of lipogenesis, which occurs with incubation in a nitrogen atmosphere, and by the absence of lipogenesis on the sides of liver buttons which were in contact with the glass vessel during incubation.

The possibility that the marginal lipogenesis was dependent on the necrosis of the adjacent surface cells seemed unlikely, for several reasons. The necrosis was not always present even when the lipogenesis was conspicuous. On the other hand, necrosis occurred often in the interior of the button and was then unassociated with lipogenesis.

Cytology: The experimental lipogenesis occurred within the cytoplasm of hepatic cells, in Kupffer cells and in the bile-duct epithelium. Although predominantly found in the marginal zone, as previously described, lipogenesis was found in bile-duct epithelium at a greater depth from the surface than in the hepatic cells, suggesting, perhaps, a less rigorous dependence of these latter cells on oxygen. A considerable penetration of lipogenesis into the liver buttons was also seen about the portal veins, presumably due to the connection of these venous spaces with the surface. Similarly, when crisscross incisions were made on the surface of the liver button, lipogenesis was found to follow the distribution of the incisions.

Several factors suggest a relationship between the presence of glycogen and lipogenesis. Both glycogen neogenesis and lipogenesis are limited to marginal zones of liver buttons incubated in vitro. Moreover, glycogen appears to be metabolized and disappears as new fat forms.‡ However, further observations negate a dependent relationship between the two. Thus, starved animals whose livers have been depleted of glycogen show as effective hepatic lipogenesis on incubation as do the glycogen-filled livers of animals which have been given glucose beforehand. It would thus seem more likely that glycogen utilization and lipogenesis in liver buttons were independent metabolic functions.

Species: Guinea pig liver and rat liver showed the same phenomenon on incubation, that is, a predominantly marginal lipogenesis, as did rabbit liver.

Chronology of events: Lipogenesis was first evident in incubated liver tissue in 3 hours, was considerable in 6 hours, and was maximal in 24 hours. Longer incubation did not increase the amount. In comparison with the cornea, lipogenesis in the liver thus occurred sooner but did not result in an increasing accumulation with prolonged incubation.

Enzyme Inhibition: Experimental lipogenesis in liver buttons was diminished by KCN in concentrations of M/1000 and abolished in concentrations of M/80. It is of some interest that the metabolic utilization of indigenous fat was also prevented by M/80 KCN. Experimental lipogenesis was also diminished by dinitrophenol in concentrations of M/1500 but was not completely abolished even with concentrations as high as M/400. This may mean that some residual energy source was available in the liver prior to the incubation with dinitrophenol.

Relation to Necrosis: The possible relationship of lipogenesis to the origin of fat in fatty degeneration has been one of the motivating interests in the present studies, and the liver seemed to be the most suitable tissue for pertinent tests. It is known from the foregoing that necrotic liver cells contain suitable substrate, presumably oleate, which will form fat in viable cells so long as serum is present. Further, it is known from the corneal study that this substrate from tissue is not readily diffusible and that the liver extract injected into tissue will form fat only in the adjacent cells.

By inference, therefore, necrotic liver tissue might be expected to induce lipogene-

‡ The glycogen disappeared entirely from the buttons during incubation, as determined by the periodic acid-Schiff-saliva test. Control buttons soaked in water at room temperature showed no reduction in glycogen content.

sis in the adjacent normal liver cells. This was found to be the case both in vivo and in vitro. Necrotizing substances, such as boiling water and acids injected into the liver of the living animal, induced within 12 hours a striking zone of fat in the hepatic cells adjacent to the area of necrosis. Freezing a portion of the liver for 10 minutes in vivo § resulted in a similar zone of fat formation just outside the necrotic area. More informative, however, were the experiments wherein the livers were removed at various times after freezing and subsequently incubated in serum. Such livers removed one hour after freezing showed no lipogenesis in the zone which had been frozen (or utilization of the indigenous fat in this area) but a zone of active lipogenesis in the hepatic cells lining the frozen zone and, of course, a marginal zone of lipogenesis in the normal portions of the liver. The same was true for livers removed three hours after freezing, but livers removed in six hours or more showed a zone of lipogenesis about the frozen area prior to incubation. It was particularly noteworthy that the fat formed in these experiments, as in the previous ones, occurred in cells that were morphologically normal and showed no evidence of degeneration even on incubation.

These experiments on freezing in vivo with subsequent incubation in vitro presented further evidence that the presence or absence of lipogenesis did not depend on glycogen. Freezing for 10 minutes completely and promptly abolished the capacity for lipogenesis, but glycogen did not disappear from the frozen zone for at least three hours.

Thus, in comparison with the cornea, liver showed an analogous lipogenesis which was serum-dependent but in which the lipogenesis occurred only at the margin of the button or adjacent to a zone of necrosis. This marginal localization appeared to depend on the availability of oxygen from the medium. Unlike cornea, liver provided its own oleate factor. As in the case of the cornea, experimental lipogenesis was inhibited by cyanide, dinitrophenol, and incubation in a nitrogen atmosphere. It did not appear to depend on the utilization of native glycogen and was not enhanced by fortification of the serum with glucose.

The fat which formed in the liver adjacent to a zone of necrosis, and which would bear the name of "fatty degeneration" in pathology, is similar to experimentally induced lipogenesis and would appear to derive from the same active process.

2. *Miscellaneous Tissue.*—Experimental lipogenesis was tested on a wide variety of rabbit tissues and on some guinea pig tissues. These tests varied in number from a single observation on some tissues to as many as 40 runs on others, depending on the interest in the particular tissue. Special attention was paid to lipogenesis in the aorta because of the possible relevance of experimental lipogenesis to atheroma formation.

The procedure consisted chiefly of excising small pieces of rabbit tissue (a few millimeters on a side) and incubating them for 24 hours in serum-oleate media and in separate vessels. Occasionally oleic acid or the sodium oleate was injected in vivo. All the experimental tissues were compared with uninjected and unincubated controls. The results obtained are listed below, arranged alphabetically according to the tissue tested, with no more than a brief mention of the findings except in selected cases of special interest. The number of separate incubations for each tissue is given in parenthesis.

Adipose Tissue (7): Observations were made on omental fat, groin fat, and fat tissue adjacent to the aorta and heart. Lipogenic fat, when present, could be distinguished from normal adipose tissue by the small size and multiplicity of globules of the former.

Injection of oleic acid into areas adjacent to adipose tissue in vivo produced a slight

§ Freezing was accomplished by having a dry ice-alcohol mixture in a test tube and applying the bowl of the test tube to the liver for 10 minutes.

lipogenesis in the proximal cells. To test lipogenesis in the true fat cells, it was first necessary to deplete them of their indigenous fat. This was done by starving guinea pigs for several days prior to removal of the tissue for incubation. It was then found that little or no fat formed on incubating for 24 hours in serum or serum oleate. It thus appeared that the factors responsible for normal deposition of fat in adipose tissue differed from those of experimental lipogenesis.

A further point of difference in the fat of adipose tissue and that of experimental lipogenesis was the failure of the former to be appreciably metabolized during incubation.

These observations contrast with those of Shapiro et al.,[7] who found a significant increase in fat content of intraperitoneal fat tissue after incubation with serum. These authors also reported a lipogenesis and take-up of fatty acids (which ones are not stated) from serum by adipose tissue of starved animals and showed that this depended on an enzymatic process that could be poisoned by cyanide of fluoride. In other words, Shapiro et al. found for adipose tissue what we find for various tissues, but what we do not find for adipose tissue. The reason for this discrepancy is not apparent, but it is noteworthy that these investigators found that the take-up of fatty acids occurred only with a critical degree of starvation (four to five days). Too little or too much starvation resulted in no fatty acid take-up.

Adrenals (2): Observations on the cortex were inconclusive because of the large amount of indigenous fat (which, like adipose fat, showed no evidence of lipolysis on incubation). The medulla showed no lipogenesis.

Aorta (26): Numerous observations were made on vascular tissue, particularly the aorta, because of the possible role of lipogenesis in atheromogenesis. Injection of oleic acid or sodium oleate into the arterial wall or adjacent to the artery of the living rabbit resulted by three days' time in only slight and inconstant lipogenesis in the adventitia and occasionally in the intima.

Incubation of aorta in serum oleate, however, induced an abundant lipogenesis of the cells of the media. As in the case of the cornea, this was oleate-dependent, with similarly optimal concentrations of 2-5 mg. of sodium oleate per milliliter of serum. Unlike the cornea, however, in which the lipogenesis was already abundant in 24 hours of incubation, that in the aorta was often not apparent until 48 hours and not abundant until 72 hours or longer. In other respects the lipogenic capacity of the aorta is identical with that of the cornea.

Atheroma consists chiefly in proliferation of the intima, and the small lipid droplets of these cells in incipient atheroma have the appearance of experimental lipogenesis. It was impossible to obtain a human aorta with atheroma sufficiently fresh to test the lipogenic capacity of these cells. In the future it is hoped to try a more crucial set of experiments, in which fibroblastic proliferation of the intima is induced prior to testing for experimental lipogenesis.

Brain (8): Incubation failed to induce experimental lipogenesis in either the white or the gray matter of the cerebrum, cerebellum, or pons. There was slight and inconstant lipogenesis in the pia but a considerable lipogenesis in the cells of the choroid plexus. This latter contained a certain amount of fat indigenously, but incubation either in serum-oleate or serum alone increased it considerably.

Bronchus (2): Mild lipogenesis was present in the epithelium and stroma.

Connective Tissue: No systematic comparison was made of lipogenesis of connective tissue in different parts of the body, but observations made in association with the other tissues showed that most connective tissue has a considerable capacity for lipogenesis and this is oleate-dependent.

Diaphragm (2): Mild lipogenesis was present in some muscle fibers, as in the case with all skeletal muscles (*vide infra*).

Esophagus (1): There was considerable lipogenesis in the epithelium and underlying

stroma but none in the smooth muscle.

Eye Tissue (other than cornea) (6): The conjunctival epithelium and sclera showed the same capacity for abundant lipogenesis as did the corneal epithelium and corneal stroma. The lens epithelium and iris epithelium similarly showed a strong capacity for lipogenesis. But the iris stroma, ciliary body, retina, and optic nerve showed no lipogenesis. The extraocular muscles showed mild lipogenesis in some fibers only.

Embryo (2): A 10 mm. rabbit embryo showed some indigenous fat in its surface ectoderm. This was greatly increased on incubation in serum-oleate but not in serum alone. The cornea of a fetus showed the same capacity for lipogenesis as the adult.

Granulation Tissue (12): Considerable lipogenesis occurred in the cells after incubation in serum-oleate or serum only.

Harderian Gland (1): No definite lipogenesis was demonstrated, but the results were difficult to interpret because of the large amount of indigenous fat. No apparent metabolism of the indigenous fat occurred on incubation.

Heart (11): A considerable number of experiments were done on the heart, in part because of their possible bearing on fatty degeneration in the heart but chiefly because early observations had indicated that when the heart was incubated in serum-oleate and stained in Sudan en bloc a peculiar crisscross pattern of lipogenesis occurred in the subendocardial muscle cells of the septum and papillary muscles of the left ventricle. No comparable lipogenesis occurred in the right ventricle or in muscle tissue other than that immediately beneath the endocardium. One of the striking features of the lipogenesis in the septum and left ventricle was the occurrence of the fat formation only in certain muscle fibers, which appeared otherwise indistinguishable in the rabbit from fibers showing no fat formation. The fat formed in the muscle cells consisted of a myriad of fine droplets scattered throughout the sarcoplasm. It depended on the presence of oleate as well as serum and was not enhanced by using muscle from animals which had been starved for several days prior to death.

The significance of these findings on the heart are not apparent to us. The peculiar distribution of muscle cells or collection of muscle cells showing lipogenesis suggested a relationship to the Purkinje system, but this could not be confirmed, for lack of means to identify this system histologically in the rabbit. It is, however, strikingly suggestive of the fatty degeneration of the left ventricle occurring with certain anemias and called, because of its patchy distribution, the "thrush-breast" or "tigroid" heart.

Hypophysis (1): Considerable lipogenesis occurred in the anterior lobe, posterior lobe, and connective tissue, all of which were oleate-dependent.

Gastrointestinal Tract (7): Little or no lipogenesis was found with 24 hours' incubation of stomach, duodenum, jejunum, ileum, colon, or appendix. Incubation reduced the staining capacity for hematoxylin, suggesting that autodigestion took place during incubation, as had been found to be the case for pancreas.

Kidney: Oleic acid injected into the kidney three days prior to sectioning showed striking lipogenesis adjacent to the zone of necrosis. The fat was present abundantly in the tubules and in the interstitial cells. These interstitial cells survived the necrosis better than the tubular cells. The glomeruli, on the other hand, showed only minimal lipogenesis.

Incubation of kidney buttons showed abundant lipogenesis in some of the tubules adjacent to the incubation media. This was not oleate-dependent. Lipogenesis also occurred in the hilar epithelium.

Thus the kidney tubules showed the same type of lipogenesis as had been found in the liver, that is, marginal fat formation which was not oleate-dependent. It is not clear, however, why the lipogenesis was limited to some of the tubules.

Leukocytes: Polymorphonuclear cells of tissue which had been injected with oleic

acid showed no fat even when the adjacent fibrocytes showed abundant lipogenesis.

Lungs (3): Incubation induced a moderate lipogenesis in certain of the cells near the surface of the tissue.‖ These cells appeared to be alveolar cells, and, unlike the liver and kidney, they did not form a continuous stratum.

Lymph Node (1): No significant lipogenesis occurred on incubation.

Mucous Membrane (1): On incubation nasal mucosa showed abundant lipogenesis, which was oleate-dependent.

Muscle (8): Incubation of muscle yielded variable results. In individual instances, a lipogenesis which was oleate-independent occurred in some muscle fibers of the vagina, uterus, tongue, diaphragm, striated muscle of the esophagus, and (as previously noted) the eye. On the other hand, no lipogenesis was induced in the smooth muscles of the trachea or esophagus. The striking feature of muscle lipogenesis was its spotty distribution. Fat formed in some fibers and not in others which were otherwise histologically indistinguishable.

Nerve (2): Little or no lipogenesis was found in the nerves of the cornea or tongue on incubation, or of the femoral or optic nerves.

Ovary (1): Abundant lipogenesis occurred on incubation in the large follicles and in the interstitial cells of the medulla, but only slight lipogenesis in the ingrowing follicles and interstitial cells of the cortex and no lipogenesis in ova. The lipogenesis was predominantly marginal and oleate-independent.

Pancreas (7): No lipogenesis was produced in the pancreas on incubation, and staining of the tissue with hematoxylin and eosin was poor after incubation. Moreover, pancreas inhibited lipogenesis in other tissue when added to the media. Presumably, this adverse effect of pancreas is attributable to the cytolytic enzymes in pancreas.

‖ In order to immerse the lung in the incubation media, the preparation was subjected to vacuum evacuation prior to incubation.

Parathyroid Gland (1): Although the parathyroid normally contains some fat, this was increased greatly on incubation. This increase, interpreted as lipogenesis, is predominantly marginal and oleate-independent.

Scar Tissue (7): Corneas scarred by prior thermal or mechanical injury showed, on incubation, lipogenesis in both the scarred tissue and the normal tissue, possibly less in the former.

Spinal Cord (1): No lipogenesis occurred in either white or gray matter.

Spleen (7): Lipogenesis occurred in the spleen but was never very marked. Injection of oleic acid into the spleen in vivo resulted in a rim of what appeared to be lipogenic fat in three days. Incubation resulted in a marginal type of lipogenesis associated with the red pulp rather than with lymphatic tissue or trabeculae. It was oleate-independent.

Testis (1): Abundant lipogenesis occurred in the interstitial cells (Leydig cells) near the surface of the incubated specimen, and some lipogenesis occurred in the connective tissue cells but none in the tubular cells.

Thyroid (1): No lipogenesis occurred on incubation of the thyroid, although observations on the same specimen showed considerable and simultaneous lipogenesis in the parathyroids.

Tongue (1): Considerable lipogenesis occurred with incubation in the epithelium, which was oleate-dependent, and some in the muscle, which was oleate-independent.

Trachea (2): Abundant lipogenesis occurred on incubation in the epithelium and in the submucosa. This was oleate-dependent.

Umbilical Cord (1): The stromal cells showed normally some fat in their cytoplasm, but this was greatly increased on incubation in serum-oleate media.

Uterus (2): Incubation induced considerable lipogenesis in portions of endometrium exposed to the medium, but no lipogenesis in crypt epithelium not in contact with the medium.

Vagina (1): Incubation resulted in abundant lipogenesis in the epithelium and some of the muscle.

Comment.—It is obvious from the foregoing that the capacity for lipogenesis varied greatly with different tissues. The factors responsible for this diversity were not entirely clear, but there did appear to be two types of reaction on incubation. These were represented by the cornea and liver, respectively. Lipogenesis in the former occurred throughout the tissue and depended on having the oleate molecule in the medium in addition to serum. In the liver type, on the other hand, lipogenesis occurred predominantly in the marginal portions of the tissue, adjacent to the medium, and did not depend on the presence of oleate added to the medium. The tissues giving the corneal type of reaction were aorta; connective tissue, including scar tissue; sclera; tracheal mucosa; tongue mucosa; nasal mucosa; umbilical stroma; hypophysis, and probably heart. Substances giving the liver type of reaction were kidney, lung, granulation tissue, ovary, choroid plexus of the brain, some muscle, parathyroid gland, and spleen. In general, but not invariably, the more parenchymatous tissues exhibited the marginal and oleate-independent type of lipogenesis. No significant lipogenesis was found with the following: adipose tissue, intestinal mucosa, nerve tissue (brain, spinal cord, retina, optic nerve, and peripheral nerve), lymph nodes, polymorphonuclear leukocytes, thyroid gland, and some muscles.

If the interpretations of the process in the cornea and liver are tenable for the other tissues, one would conclude that the tissues giving a liver type of reaction had sufficient oleate substrate within their parenchyma for lipogenesis and depended on an oxygen supply being more readily available to the individual cells than is the case with tissue giving the corneal type of reaction.

It is of some further interest that there is no correlation between cells containing some types of indigenous fat (adipose tissue and adrenal cortex) and the capacity for experimental lipogenesis. This, together with the fact that these native lipids, unlike lipogenic fat, were not metabolized on incubation, led to the inference that depot fat formation (presumably physiologic) and experimental fat formation (presumably pathologic) are distinct processes.

Summary and Conclusions

Experimental lipogenesis was the result of an intracellular enzymatic process having an optimal pH range about neutrality. It was inhibited by cyanide, dinitrophenol, and anoxia.

The capacity for lipogenesis was preserved for several days at temperatures of 2-5 C. It resisted lyophilization and radiation (15,000 rep) but was destroyed by immersion of the tissue in distilled water in a matter of minutes or by mechanical pressure.

Tissues varied in their capacity for, and manner of, lipogenesis, but those capable of inducing lipogenesis may be classified into two types, called liver type and corneal type, respectively. Excised liver buttons differed from corneal buttons in showing lipogenesis only along the exposed margins of the tissue (apparently a function of oxygen availability) and in requiring no addition of oleate to the medium. A type of lipogenesis similar to that of liver occurred with kidney, lung, granulation tissue, ovary, choroid plexus of brain, some muscle, parathyroid gland, and spleen. On the other hand, a type of lipogenesis similar to that of cornea (diffuse throughout tissue and dependent on addition of oleate to the medium) occurred with aorta, connective tissue, sclera, and some mucous membranes. No experimental lipogenesis occurred with adipose tissue, nerve tissue, lymph nodes, polymorphonuclear cells, and thyroid gland.

It is inferred that the process of fat formation with necrosis, the so-called "fatty degeneration," and atheromogenesis result from a dynamic and oleate-induced synthesis of fat similar to the lipogenesis here reported, rather than from the liberation of

masked fat or lipid phagocytosis of preformed fat, as is generally believed.

Further studies will be directed toward chemical identification of the fat formed.

243 Charles St. (14).

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Massive Calcification of the Myocardium of Unknown Origin

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Calcification of the myocardium has been described many times. Massive calcification has been described with less frequency. The following case is one of massive calcification of the interventricular septum of the heart of a middle-aged man for whose heart disease no definite cause could be established.

Report of a Case

A 41-year-old Puerto Rican man was seen at the medical outpatient clinic for the first time on Aug. 8, 1955, with complaints of severe dyspnea, orthopnea, dizzy spells, and frequent epigastric pain unrelated to meals. At 27 years of age he was hospitalized in Puerto Rico because of chest pain, palpitation, dyspnea, nausea, vomiting, and ankle edema; he was told of heart disease and apparently was treated with quinidine. At the age of 36 a diagnosis of enlarged left heart, a conduction abnormality, ventricular tachycardia, and cardiac insufficiency was made during a similar episode. Rheumatic fever, characterized by polyarthritis, chorea, and carditis, was stated to have occurred at the ages of 7, 14, and 33 years. In the summer of 1954 he was treated briefly for congestive heart failure, but no adequate description of his course at that time was available. There was no history of other illnesses.

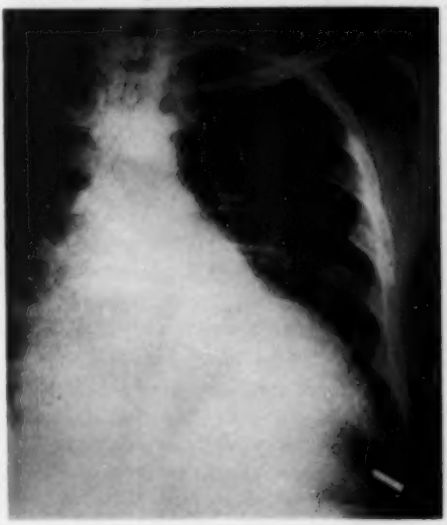
Physical examination revealed a blood pressure of 140/60 mm. Hg, a pulse rate of 64 per minute with an irregular rhythm, an enlarged heart, a systolic murmur and a diastolic sound at the apex, a liver that was slightly enlarged and tender, and 2+ pretibial edema.

A roentgenogram showed an enlarged heart and pericardial calcification (Fig. 1*A* and *B*). The initial electrocardiogram revealed a normal sinus rhythm with His-bundle premature systoles and coupling, and intrinsicoid deflections in Leads V_1 and aV_L of 0.08 second each, interpreted as bilateral bundle-branch block. A later tracing showed the additional finding of runs of His-bundle tachycardia.

On the basis of the history and physical findings, the diagnosis of rheumatic heart disease with congestive heart failure was made in the clinic. For six weeks the patient received digitalis, with initial improvement. This medication was then stopped, and mercurial diuretics were used to control the signs and symptoms of failure. On Oct. 27 quinidine sulfate, 0.2 gm. four times a day, was started in an attempt to control the cardiac rhythm.

The patient was admitted to the hospital on Nov. 1 because of dyspnea, chest pain, dizziness, palpitation, headache, nausea, and vomiting for the preceding 20 hours. The blood pressure was 120/70 mm. Hg and the pulse rate 200 per minute. The heart was enlarged, and the lung fields were clear. The remainder of the physical examination was unremarkable. An electrocardiogram revealed a ventricular tachycardia of 156 per minute. Quini-

Fig. 1A.—Posteroanterior view of the chest. An area of calcification is present at the apex of the heart. There is generalized cardiac enlargement.



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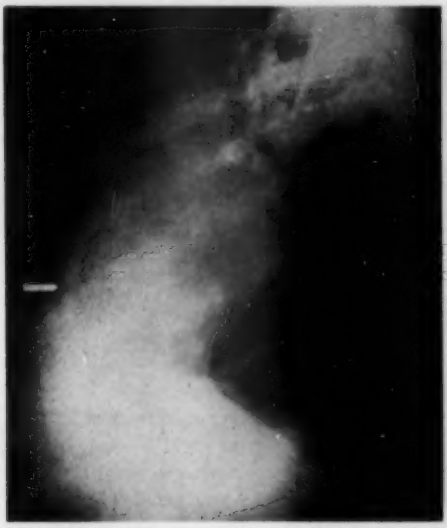

Fig. 1B.—Lateral view of the chest. A curved dense shadow, convex anterior, is present in the anterior portion of the heart shadow. The significance of this density, which represents calcification, was not appreciated until the x-rays were reexamined after the autopsy.

dine was stopped, and, after a few hours of careful observation, 750 mg. of procainamide (Pronestyl) hydrochloride U. S. P. was administered intravenously. There was temporary slowing of the heart rate, but no change in the form of the electrocardiographic deflections. Oral procainamide, changed to 250 mg. of intramuscular procainamide every three hours because of vomiting, maintained the rate at about 150 per minute, with no change in the basic rhythm. Laboratory studies revealed the following values: hemoglobin 16 gm. per 100 cc.; white blood count 13,500, with a normal differential count; a negative urinalysis, with a specific gravity of 1.020; blood urea nitrogen 37 mg. per 100 ml.; cephalin flocculation 3+.

During the following week increasing doses of procainamide hydrochloride, up to 500 mg. intramuscularly every one and a half hours, slowed the rate at times to 120 per minute, with no change in the focus of the tachycardia. On the fourth day of hospitalization a gallop rhythm was heard for the first time. On the ninth day the patient appeared jaundiced, and further laboratory studies revealed the following: icteric index 95; cephalin flocculation 4+; total protein 5.6 gm. per 100 cc., with albumin 3.6 gm. and globulin 2.0 gm. per 100 cc.; alkaline phosphatase 7.5 Bodansky units per 100 cc. Bile was present in the urine. Because of the jaundice and the failure of the rhythm to respond to procainamide, this drug was discontinued on Nov. 14. At the time, a gallop rhythm was still present, a few rales were audible at the bases of the lungs, and the heart rate was increased to 160 per minute.

In a further attempt to alter this ventricular tachycardia, quinidine was started again, 0.4 gm. every four hours. An electrocardiogram taken on Nov. 16, after this therapy had been initiated, revealed the presence of a ventricular tachycardia, again at a rate of 156 per minute, but this time from a different focus. During the following eight to nine days the lungs cleared and the heart rate slowed to 107 per minute. There was still no evidence of A. V. conduction, and the ventricular tachycardia persisted. On Nov. 25 the patient appeared to be out of contact with his environment and attempted to leap from the window. An electrocardiogram taken on that day showed a marked change—a complete heart block with a widened QRS complex and a ventricular rate of 75 per minute. He died on the same day.

Postmortem examination was performed seven hours after death. The body was that of a well-developed man 4 ft. 11 inches (150 cm.) tall, with intensely jaundiced skin and sclera. The abdominal, pleural, and pericardial cavities were normal except for a few fibrous adhesions in the right pleural space. The heart weighed 450 gm. The epicardium showed an area 3 by 1.5 cm. over the anterior wall of the left apex where a semisolid zone of necrosis continuous with and partially covered by calcium was present. On cutting into this area, a moderate amount of pinkish-white, "cheesy" material was found extending into the interventricular septum. Farther in the septum, a large fibrocalcific plaque was present, measuring 6×5 cm. and 0.3-0.4 cm. in thickness and extending to within 1 cm. of the aortic valve (Fig. 2*A* and *B*). The septum itself bulged slightly into the right ventricle. Other areas of the myocardium were unremarkable, and the valves appeared normal. The coronary arteries were all patent and showed only minimal atheromatous changes. The thickness of the left ventricle was 1.1 cm. and that of the right ventricle 0.1-0.2 cm. The aorta contained minimal lipid streaking in its abdominal portion. A thrombus occluded a large pulmonary artery to the left lower lobe, with an infarct distal to it. The liver was jaundiced, weighed 1280 gm., and the lobular architecture was accentuated. The spleen weighed 200 gm. and was firm and rubbery. A chronic 2×1 cm. ulcer was present in the first portion of the duodenum, penetrating into the pancreas. The mucosa of the urinary bladder was hemorrhagic and ragged, with yellow encrustations that could not be scraped off. The pancreas, adrenal glands, extrahepatic biliary system, neck organs, and kidneys were essentially normal.

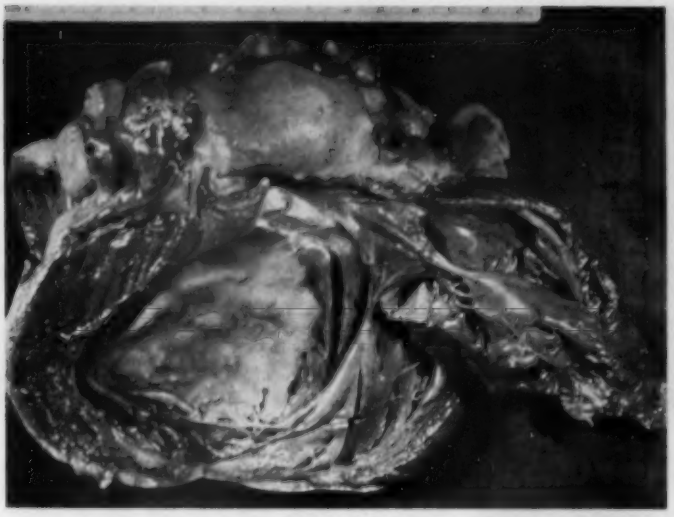

Fig. 2A.—The interventricular septum as seen from the left ventricle. The large white area consists of fibrous tissue and calcium deposits.

The microscopic examination revealed the following: Sections from the left ventricle showed muscle hypertrophy and mild focal replacement of fibers by fibrous tissue. Sections from the septum revealed almost complete replacement of the muscle fibers by dense, acellular fibrous tissue, large areas of calcification, and a few small areas of ossification. The few muscle fibers present were hypertrophied. Another section from the septum revealed the above features plus dense aggregates of neutrophils, lymphocytes, and a few plasma cells, often surrounding congested blood vessels. A large amount of amorphous basophilic material was present, at the periphery of which there was some tendency toward palisading of mononuclear cells with an occasional giant cell. A section from the "puriform" abscess of the apex showed more of this amorphous basophilic material, surrounded by a similar inflammatory response, fibrous tissue,

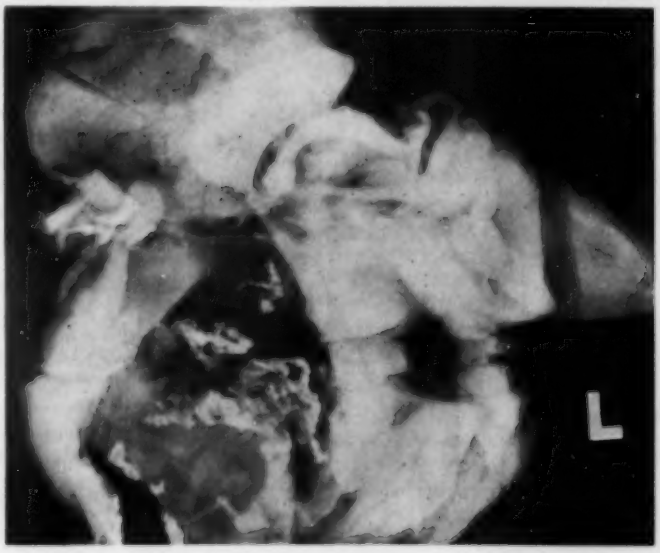

Fig. 2B.—An x-ray of the same view as that in Figure 2A, after blocks were removed for sections. The extent of the calcification can be easily appreciated throughout the interventricular septum.

and deposits of calcium. The mitral valve leaflet was normal. Special stains for fungi, bacteria, and amyloid were all negative. The lung showed changes of chronic passive congestion with hemorrhagic infarction. The liver was the site of chronic passive congestion with several small granulomas, one of which contained a schistosome ovum. There was chronic passive congestion of the spleen. In the pancreas there was a mild increase in fibrous tissue, with several small areas of fat necrosis in surrounding adipose tissue. One adrenal gland had an organizing thrombus in the central vein, with a large area of hemorrhagic necrosis of the cortex and medulla. The urinary bladder was greatly thickened by congestion, hemorrhage, and fibrous tissue, with a mild infiltration by lymphocytes, plasma cells, and occasional large mononuclear cells. A section from the duodenum revealed a chronic ulcer. Sections from the kidneys, thyroid, parathyroid, and bone were normal.

Summary of Patholigical Findings.—Massive calcification of interventricular septum of heart with "puriform" degeneration; chronic passive congestion of lungs, liver, and spleen; thrombus in pulmonary artery with infarction of left lower lobe of lung; schistosomiasis of liver; chronic pancreatitis with fat necrosis; thrombosis of central vein of adrenal gland with recent infarction; cystitis of unknown type.

Comment

In 1924, Scholz,[1] in a discussion based on previously reported cases of calcification of the myocardium, summarized his findings by classifying such calcification as being on the basis of previous myocarditis, obliteration of the coronary vessels, sepsis, or metastatic calcification. In a similar review, Diamond,[2] in reporting a case of extensive calcification of the myocardium in a premature infant, attributed calcification to vascular disease, or to true inflammatory conditions, or to toxic necrosis, either infectious or noninfectious. The lesions in those cases reported in both reviews varied from those of microscopic size to large areas observed grossly. Since then, there have been several reports of massive calcification of the myocardium, the etiology of occlusive coronary disease being well recognized in the majority of cases.[3-10] However, there have been occasional reports of massive calcification in the absence of any coronary disease. Edelstein [11] discussed the findings in an 11-year-old boy with massive calcification and bone formation in the left ventricular myocardium. The history revealed an episode of scarlet fever at 6 years and rheumatic fever at 7 years of age. The author suggested the possibility that the degenerative changes in the myocardium preceding the calcification were due to diphtheria or infection with Hemophilus influenzae. Van Buchem [12] reported a case of extensive calcification in the endocardium and subendocardium of the wall of the left ventricle of an 18-year-old woman. On the basis of the history, the author raised the possibility of endocardial injury following physical exertion as an etiologic factor. A case of extensive calcification of the myocardium involving the left ventricle, left auricle, and interventricular septum was reported by Ernstene and Hazard,[13] occurring in a 25-year-old woman who died in congestive heart failure. The history stated that she had scarlet fever 16 years and pneumonia 4 years prior to her death. The deposition of calcium was considered to have occurred during the healing stage of a severe toxic or septic myocarditis. These three cases, like the case that is being reported, had no evidence of coronary artery disease.

In order to establish this case as one of massive myocardial calcification of unknown etiology, it is necessary to examine all etiologic possibilities in the light of what is known about this patient. A vascular origin can be excluded by the absence of a significant amount of any type of coronary artery disease. A congenital abnormality of the coronary vessels was not present.[14] The possibility of a previous myocarditis has to be considered. The history that was obtained mentions only rheumatic fever.

None of the usual stigmata of old or recent rheumatic endocarditis or myocarditis were seen at autopsy. Schistosomiasis of the liver was found. Although schistosomal myocarditis does occur,[15] there was no evidence that could incriminate this as the cause. Myocardial necrosis secondary to an infectious disease or to specific exogenous toxins[16,17] might be followed by calcification. No history of such etiologic factors was obtained. Furthermore, one might expect myocarditis of this type to produce a more diffuse lesion, rather than limit itself to the septal myocardium. The character of the lesion, the absence of any calcification in other organs, and the presence of normal kidneys, parathyroids, and bone make it extremely unlikely that this case represents one of metastatic calcification.[18] It must be assumed, then, that myocardial necrosis of unknown origin preceded the calcification in this case.

The electrocardiograms indicate impairment of interventricular conduction, probably bilateral, and augmentation of impulse initiation in the ventricles. With the degree of morbidity found at necropsy, and with its distribution so vitally placed, one can readily understand the reason for these abnormalities and their refractoriness to treatment.

It is conceivable that with a more extensive radiologic investigation of the "pericardial calcification" it might have been possible to appreciate the area of septal calcification. The radiological differential diagnosis of pericardial and myocardial calcification has been discussed by Brean, Marks, Sosman, and Schlesinger.[9]

Summary

A case of massive calcification of the septal myocardium, unsuspected clinically, is presented. The literature is reviewed briefly, particular attention being paid to those cases in which coronary artery disease was not an etiologic factor. Upon review of the pathological and clinical findings in this case, it was felt that the cause for the myocardial calcification could not be determined.

Dr. Marvin Kuschner and Dr. Charles E. Kossmann gave help and criticism in the preparation of this paper. The photographs were taken by Mr. Robert Waldeck.

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Studies on Connective Tissue

I. The Polysaccharides of the Human Umbilical Cord

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Introduction

An adequate description of the physiologic role of ground substance depends on the correlation of information obtained by chemical studies, histochemical methods, physicochemical description, and studies of the metabolism of these materials. The anatomic and cytologic location and mechanism of formation are important in the correlation of what may appear to be nonrelated information.

In the mature umbilical cord two acidic polysaccharides, hyaluronic acid and chondroitin sulfate C, have been isolated. The demonstration of mast cells in this tissue suggests that heparin is present.[1-6] The studies reported here have been carried out on umbilical cords from 6-week-old fetuses through full-term infants. It was felt that this tissue would give a suitable survey of the embryonic development of the ground substance, as well as the anatomic location of individual polysaccharides.

The pH-ionic strength dependence of the metachromatic reaction, the periodic acid-Schiff reaction, a variety of enzymatic studies, and the response of these to sulfation of the umbilical cords localized hyaluronic acid, chondroitin sulfate, and heparin in definite sites. Differences in the reactions of the cords with age, determined by these methods, suggest that part of the synthesis of hyaluronic acid and/or chondroitin sulfate is extracellular, while the synthesis of heparin is completely intracellular.

Experimental Methods

Mature and immature human umbilical cords from 6-week-old fetuses through full-term infants were selected for study. Formalin, Carnoy fluid, and 95%, 80%, and 75% ethanol fixatives were employed. To facilitate fat extraction, tissue also was fixed in Bouin's solution.[7] Multiple frozen sections, averaging 15μ in thickness, were cut from each of the cord specimens in the various fixatives. These were mounted on glass slides with egg albumin as the adhesive, and air-dried. The younger cords were graded as to age. Identical procedures were applied to mature and immature cords. Paraffin sections treated in the usual manner were also studied. These sections were thinner, in the order of 5μ to 8μ.

For the studies of metachromasy,* toluidine blue solutions, 1.5×10^{-4} molar† were prepared in various buffers from pH 2.0 to 7.5 at intervals of 0.5 unit and at ionic strengths calculated to be 0.0025, 0.01, and 0.1.[10] The ionic-strength increments were made by addition of sodium chloride; the sodium ion was the only metal cation. While the buffering capacity was diminished at the lower ionic strength, the pH adjustments that were required were small and did not significantly affect the calculated ionic strength. In addition, solutions of the dye of the above concentration were made in distilled water and the pH adjusted to compare with the corresponding buffers with either HCl or NaOH. Ionic-strength adjustments were made

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* Metachromasy in this paper is defined as the change in absorption of toluidine blue from its normal absorption in the red to that in the blue. This is visually distinguished as a shift in color from the blue to the red. All observations of metachromasy reported in this paper were made in aqueous media.[8-9]

† Certified stain, National Aniline Division, Allied Chemical and Dye Corporation, Certification No. NU-12.

with NaCl. The results obtained by both methods were identical when fresh solutions of the latter were used. Buffers were generally employed, since the pH changed with age in the nonbuffered solutions.

The frozen sections were dialyzed against the appropriate buffer for 2 hours and then stained for 10 and 20 minutes. Staining time longer than 20 minutes did not affect either the intensity or the color. The paraffin sections were treated in the same way after hydration. All tissue sections were rinsed quickly in several changes of buffer after staining and allowed to air-dry, or were examined microscopically immediately. For those sections which were air-dried, rehydration was accomplished by immersion of the section in the appropriate buffer for several seconds. The results of the two procedures were identical.

Frozen sections were air-dried in an oven at 37 C for 24 hours. The slides were sulfated at 0 C for five minutes in a 1:1 solution mixture of concentrated sulfuric acid (96.8%) and glacial acetic acid (99.9%). They were rinsed in glacial acetic acid, washed in running tap water for 10 minutes, and rinsed in distilled water. Then the slides were dialyzed for two hours in the appropriate buffers and stained in the corresponding solutions of toluidine blue.

The periodic acid-Schiff reaction was carried out according to the Hotchkiss-McManus technique.[11] This was done also after extraction of the tissue sections fixed in Bouin's solution with pyridine at 60 C for 24 hours, after sulfation, and after treatment with amylase or testicular hyaluronidase by the method described by Pearse.[9] The enzyme-treated tissues were also stained with toluidine blue.

Alkaline or acid hydrolysis with 1 N solutions of NaOH, H_2SO_4, HCl, HNO_3, and HIO_4 was carried out from five minutes to six hours prior to staining with toluidine blue and the application
G 4689, National Institutes of Health.
of the periodic acid-Schiff test. Sections were stained with Sudan IV and Sudan black before and after extraction with pyridine.[9]

Results

The metachromasy of Wharton's jelly in both the mature and the immature cords was essentially the same except in the lowest age group, of 6 to 7 weeks. There was no metachromatic staining in Wharton's jelly below pH 4.0 at any ionic strength. Metachromasy first appeared at pH 4.0, $\gamma/2 = 0.0025$, as a pink hue in the interfibrillar regions. The reaction was uniform without concentration in any particular area of the cord. From pH 4.5 to pH 6.5 it became more intense for a given ionic strength, but was less intense at higher ionic strengths. In this range a blue component became prominent, which resulted in a change from pink to reddish violet. This color persisted through pH 6.5 to pH 7.5 but was less intense. In the young cords, 6 to 7 weeks, the presence of metachromatic material was variable; in some of the younger cords no, or very slight, metachromasy was observed. In relationship to age, metachromasy appeared first in Wharton's jelly, next in mast cells, and finally in the vessels.

Sulfation of the cords produced a metachromatic reaction in both term and immature cords, but this effect diminished as the age of the cords increased. This procedure induced metachromasy in Wharton's jelly at pH 2.0 to pH 4.0 and augmented metachromasy from pH 4.0 to pH 7.5 at all ionic strengths. In younger cords, where metachromasy could not be demonstrated by the usual technique, the sulfation procedure produced a distinct pink metachromatic reaction in Wharton's jelly at pH 2.0, which persisted throughout the pH-ionic strength range. The violet color was not observed.

The periodic acid-Schiff reaction was applied in an effort to determine whether the PAS-reactive sites were identical with the metachromatic sites, before and after sulfation. The extracellular fibers of the mature and immature cords showed an intense periodic acid-Schiff positivity. The interfibrillary spaces were negative or only faintly positive. This was in contrast to the interfibrillar location of the metachromatic material. Sulfation did not affect the PAS reaction. The positive material in this area is probably not related to a lipid, since the Sudan black and Sudan IV procedures were negative in this site.

Incubation of sections with hyaluronidase reversed the metachromatic reaction in Wharton's jelly. There was no effect on the PAS reaction. Treatment with amylase did

not change the metachromasy or the PAS staining of the intercellular fibers, but did reverse the PAS reaction of Wharton's jelly when present.

The arteries of the mature cord were metachromatic at pH 2.0 at $\gamma/2=0.0025$ only in the inner one-third of the wall of the vessels.[5,6] The color was bright red. As the pH was increased at $\gamma/2=0.0025$ and $\gamma/2=0.01$ the metachromasy gradually appeared throughout the arterial wall. This staining was present throughout the wall at pH 4.0 and persisted to pH 6.5 at these ionic strengths. The maximal staining of the arteries occurred at pH 5.5, $\gamma/2=0.0025$. At $\gamma/2=0.1$ the intensity was attenuated. As the pH was raised from 6.5 to 7.5, $\gamma/2=0.01$, and $\gamma/2=0.1$, the metachromatic staining was diminished.

In contrast to the arteries of the mature cords, those of the immature cords had a different metachromatic staining. In the group from 8 to 12 weeks, the metachromasy was confined to the pH range from 6.0 to 7.5, and was more intense at the lowest ionic strength and absent at $\gamma/2=0.1$. The reaction was uniform throughout the vessel wall, without indication of the subendothelial concentration of metachromatic material comparable to that found in the arteries of the mature cord under the same conditions. As the cords increased in age, metachromasy was present throughout the pH range from 4.0 to 7.5, but without subendothelial concentration. Beginning at about five months the subendothelial concentration of metachromatic material was distinct in the pH range from 2.0 to 4.0.

In the venous wall of the mature cord the distribution and the pH-ionic strength dependence of metachromasy was different from that found in the arteries. From pH 2.0 to 4.0 there was not metachromatic staining. Throughout the pH range from 4.0 to 7.5 at all ionic strengths, the most intense reaction was in a narrow band immediately beneath the endothelium of the vessel. The remainder of the vessel wall showed a diffuse metachromatic staining of lower intensity than the subendothelial region and was most intense at pH 5.0-5.5. As in the case of the arteries, the color was red throughout, without a blue component. The metachromatic properties of the immature vein were generally similar to those of the 8- to 12-week arteries.

Sulfation produced several important changes in the vessels of both the mature and the immature cords. In the outer portions of the walls of the arteries and throughout the venous walls, sulfation produced faint metachromasy in the pH range from 2.0 to 4.0. Above this range it was difficult to identify any augmentation of the metachromatic reaction, save for those cords where the initial reaction before sulfation was confined to the pH range from 6.0 to 7.5. The intensity of the reaction produced by this procedure diminished as the age of the cords increased. In those cords where no evidence of metachromasy was found in the vessels, sulfation failed to produce a metachromatic reaction.

The PAS reaction in the arteries and veins in the mature and immature umbilical cords were the same. The positive material was found in the muscle cells and on fibers. The location of this material did not correspond to the metachromatic staining materials observed before or after sulfation. The possibility that the PAS-positive reaction indicated a lipid fraction was ruled out by treatment of the Bouin fixed sections with pyridine at 60 C for 24 hours. The PAS reaction was not affected. Incubation with hyaluronidase did not alter the PAS reaction, but destroyed the metachromasy. Amylase digestion reversed the PAS reaction in the muscle cells of the arteries and veins, but the metachromatic areas were not affected. Sulfation had no effect on the PAS reaction.

In the mature cords mast cells stained metachromatically over the entire pH range at all ionic strengths. The staining of these cells was characterized by a dark-red cytoplasm. Discrete granules could not be identified.[12] The metachromasy was independent

of the method of fixation and sulfation, but increased in intensity at the higher pH values and at the lower ionic strengths. In the younger cords below 12 weeks, mast cells were only occasionally identified. In these cords metachromatic staining of the cytoplasm was seen only in the pH range from 6.0 to 7.5. After sulfation of these younger cords, cells with metachromatic cytoplasm were numerous and stained throughout the pH range.

The mast cells in both the mature and the immature cords were PAS-positive. Below 12 weeks, where only a rare mast cell could be demonstrated by metachromasy, it was possible to demonstrate a greater number of cells which were PAS-positive. These correspond cytologically to the metachromatic cells produced by the sulfation technique. The PAS reaction was not affected by sulfation. Sudan black-positive granules were identified in the cytoplasm of the mast cells. These could be removed by pyridine extraction without affecting the PAS reaction. Hyaluronidase and amylase had no effect on either metachromatic staining or PAS reaction.

Mild alkaline or acid hydrolysis with 1 N solutions of NaOH or H_2SO_4, HCl, HNO_3, and HIO_4 markedly diminished the metachromatic and PAS-staining areas, depending on time of hydrolysis.

The effect of acid and alkaline hydrolysis on these procedures was in striking contrast to that of the sulfation technique. Consequently, it is unlikely that any significant hydrolysis occurs in the sulfation technique.

The metachromasy of any of the tissue sections at a given pH and ionic strength could be reversed by redialyzing and staining at another pH and ionic strength. This resulted in a shift of the staining to that found normally for the second pH and ionic strength.

Comment

Based on the physical and chemical behavior of the connective tissue polysaccharides in vitro, several tentative conclusions may be drawn from the above results. At least three acidic polysaccharides have been identified and appear localized in definite areas of the umbilical cord. The reactions indicate differences of the polysaccharides in the youngest cords from those of the mature cords. Some suggestions concerning the synthesis of these polysaccharides can be proposed.

The pH dependence of the metachromatic staining of Wharton's jelly in cords above 8 weeks was consistent with a carboxylated polysaccharide. The appearance of the metachromasy at pH 4.0 or above is the range expected with hyaluronic acid.[13] This is based on the dissociation constants of the carboxyl groups of hyaluronic acid. The pink hue of the interstitial spaces at pH 4.0 to 4.5 was suggestive of a single substrate interacting with the dye. The change in the metachromatic color from pink to reddish violet as the pH increased indicated an additional interaction of the dye with some nonmetachromatic material, possibly protein.[14] The decrease in intensity of the reaction at pH 6.5 to pH 7.5 cannot be adequately explained.

The complete absence of metachromasy in immature cords is indicative of the unavailability of consecutive binding sites[15] or lack of a metachromatic polysaccharide. The appearance of metachromasy in Wharton's jelly after sulfation shows that polysaccharides are probably present, but apparently not sufficiently carboxylated or sulfated to give the metachromatic reaction. The possibility that either the carboxyl or the sulfate groups are covalently bound to another group must be considered. It is probable that synthesis of the polysaccharide is incomplete. Once metachromasy appears, there is much less effect by sulfation. This suggests that a small amount of the polysaccharide in the older cords is similar to that in the youngest of the immature cords. The completion of the synthesis of the polysaccharide in an extracellular location is suggested by these findings.

The reversal of the metachromatic reaction in Wharton's jelly by hyaluronidase is consistent with hyaluronic acid or chondroitin sulfate C. The pH at which the staining of this material first appears indicates hyaluronic acid, and not chondroitin sulfuric acid.

The PAS reaction is only faintly positive in the metachromatic areas of Wharton's jelly and is probably glycogen, since amylase digestion reverses the reaction. Lipid in this site is unlikely with the negative reactions with Sudan IV and Sudan black.

On the basis of the similar pH and ionic strength dependence of metachromasy and the effect of sulfation, it would appear that the metachromatic material in the outer one-half to one-third of the walls of the arteries and throughout the wall of the vein is probably identical with that in Wharton's jelly. Treatment with testicular hyaluronidase coupled with chemical isolation [1] gives support to the location of hyaluronic acid in the vessel walls, as well as in Wharton's jelly. From this it seems that sulfated polysaccharides are not a significant component in these areas, if present at all.

In contrast to the outer layers of the walls of the arteries the subendothelial zone stained at pH 2.0 and $\gamma/2=0.0025$, consistent with a lower pK_a of the sulfuric acid groups in chondroitin sulfate. Chondroitin sulfuric acid has been isolated from the umbilical cord.[1] Carboxylated polysaccharides appear in the vessel walls first, and the sulfation process occurs later, as indicated by the pH and ionic-strength dependence.

In contrast to the difference in the metachromatic reaction of artery and vein in the mature cord, the vessels of the immature cord were similar to each other. In arteries and veins metachromatic staining of the young cords below 12 weeks was confined to the pH range from 6.0 to 7.5 and was present only at low ionic strengths. This substrate probably is bound in such a manner that sites are not available for the metachromatic reaction except in a very narrow pH range. The gradual acquisition with age of the same staining characteristics as that of the mature vessels may represent a gradual build-up of metachromatic material.

The fact that metachromasy is usually augmented by sulfation indicates that the polysaccharide present is neither completely carboxylated nor sulfated. The similar pH-ionic strength dependence in the immature vessels and Wharton's jelly is in keeping with the behavior of hyaluronic acid. The failure of sulfation to affect those vessels where metachromasy was absent is best explained by incomplete synthesis of the polysaccharide. It also shows a later synthesis of sulfated polysaccharide in this site than in Wharton's jelly.

The PAS reactions in the arteries and veins were similar. The major portion of the PAS-positive material was in the muscle cells and fibers. Little or no PAS-positive material could be demonstrated in those sites which were metachromatic. The material within the muscle cell cytoplasm was removed by amylase, consistent with glycogen. Hyaluronidase did not affect the PAS reaction of the vessels but reversed the metachromatic response. The pH behavior was also consistent with chondroitin sulfate. Lipids can be excluded on the basis of the Sudan stains.

As has been previously described, mast cells in the umbilical cord were found.[2,12] Consistent with a sulfated polysaccharide, probably heparin,[2,16,17] these cells were metachromatic over the entire pH and ionic-strength range used and were not affected by sulfation or testicular hyaluronidase digestion. In cords 10 weeks of age and younger, what appear cytologically to be mast cells were few in number or absent. Where metachromatic staining was observed in these cells, it was confined to the pH range 6.0-7.5. This suggests the combination of the substrate with another material. Sulfation of cords of this age group produced metachromasy in the cytoplasm of these and additional cells throughout the

General Survey of Histochemical Findings

			pH Dependence of Metachromasy in Untreated Cords	Metachromasy Following Sulfation	Metachromasy Following Hyaluronidase Digestion	Metachromasy Following Amylase Digestion	Periodic Acid-Schiff Reaction in Untreated Cords in the Metachromatic Areas	PAS Reaction Following Sulfation in Metachromatic Areas	PAS Reaction Following Hyaluronidase in Metachromatic Areas	PAS Reaction Following Amylase in Metachromatic Areas
Wharton's jelly	6-7 wk. of age		Neg. to faint	Pos. 2.0-7.5	Neg.	Neg.	Neg. or faint	No change	No change	Neg.
	8 wk. Term		Pos. 4.0-7.5	Pos. 2.0-7.5	Neg.	Pos. 4.0-7.5	Neg. or faint	No change	No change	Neg.
	Term		Pos. 4.0-7.5	Pos. 2.0-7.5	Neg.	Pos. 4.0-7.5	Neg. or faint	No change	No change	Neg.
Artery intercellular material	8 wk. of age		Neg.	Neg.	Neg.	Neg.	Neg. or faint	Neg.	Neg.	Neg.
	8 wk. 12 wk.		Pos. 6.0-7.5	Pos. 2.0-7.5	Neg.	Pos. 6.0-7.5	Neg. or faint	Neg.	Neg.	Neg.
	12 wk. 5 mo.		Pos. 4.0-7.5	Pos. 2.0-7.5	Neg.	Pos. 4.0-7.5	Neg. or faint	Neg.	Neg.	Neg.
	5 mo. Term	Subendothelial area	Pos. 2.0-7.5	Pos. 2.0-7.5	Neg.	Pos. 2.0-7.5	Neg. or faint	Neg.	Neg.	Neg.
		Remainder vessel wall	Pos. 4.0-7.5	Pos. 2.0-7.5	Neg.	Pos. 4.0-7.5	Neg. or faint	Neg.	Neg.	Neg.
	Term	Subendothelial area	Pos. 2.0-7.5	Pos. 2.0-7.5	Neg.	Pos. 2.0-7.5	Neg. or faint	Neg.	Neg.	Neg.
		Remainder vessel wall	Pos. 4.0-7.5	Pos. 4.0-7.5	Neg.	Pos. 4.0-7.5	Neg. or faint	Neg.	Neg.	Neg.
Vein intercellular material	8 wk. of age		Neg.	Neg.	Neg.	Neg.	Neg. or faint	Neg.	Neg.	Neg.
	8 wk. 12 wk.		Pos. 6.0-7.5	Pos. 2.0-7.5	Neg.	Pos. 6.0-7.5	Neg. or faint	Neg.	Neg.	Neg.
	12 wk. Term		Pos. 4.0-7.5	Pos. 2.0-7.5	Neg.	Pos. 4.0-7.5	Neg. or faint	Neg.	Neg.	Neg.
	Term		Pos. 4.0-7.5	Pos. 4.0-7.5	Neg.	Pos. 4.0-7.5	Neg. or faint	Neg.	Neg.	Neg.
Mast cells	12 wk. of age		Pos. 6.0-7.5	Pos. 2.0-7.5	Pos. 6.0-7.5	Pos. 6.0-7.5	Pos.	Pos.	Pos.	Pos.
	12 wk. Term		Pos. 2.0-7.5	Pos. 2.0-7.5	Pos. 2.0-7.5	Pos. 2.0-7.5	Pos.	Pos.	Pos.	Pos.

* Metachromatic reactions in this chart were observed at $\gamma/2=0.0025$.

pH range of 2.0-7.5. The results suggest the presence of some precursor of the metachromatic polysaccharide component of the more mature mast cells. This material is very likely different from the PAS-positive material present in all mast cells and is probably not lipid. From the differences in the metachromatic staining properties of mast cells with age, it would appear that the total synthesis of heparin is intracellular. This is in contrast to hyaluronic acid and chondroitin sulfuric acid in the umbilical cord, where part of the total synthesis is extracellular.

Summary

The location of the anionic polysaccharides of the human umbilical cord from the early fetus to the term infant was studied by histochemical methods.

Hyaluronic acid is present in Wharton's jelly, the vein wall, and the outer portion of the wall of the arteries. Chondroitin sulfuric acid is found in the inner layers of the wall of the arteries. Heparin or a heparin-like material is present in the mast cells. In addition, glycogen is located in the muscle cells of the vessel, and to a much less extent in Wharton's jelly, and the interfibrillary spaces of the vessels.

Differences in the reactions of immature cords and mature cords strongly suggest that at least a part of the synthesis of hyaluronic acid and chondroitin sulfuric acid is extracellular, while that of heparin is completely intracellular.

Miss Marie Callahan and Miss Doris Huxley assisted in this study.

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Studies on Ochronosis

I. Report of Case with Death from Ochronotic Nephrosis

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Ochronosis is an inborn error of phenylalanine and tyrosine intermediary metabolism. The gene controlling the degradation of homogentisic acid, a breakdown product of phenylalanine and tyrosine, is lacking. This defect results in the accumulation of homogentisic acid in the extracellular fluid, and the oxidation of homogentisic acid in the presence of alkali produces the dark urine characteristic of the disease.[1] For some unknown reason homogentisic acid selectively localizes in cartilage, accounting for the black cartilaginous discoloration that is one of the cardinal features of ochronosis. Although the disease may not necessarily shorten life, it causes disabling arthritis, and it is often accompanied by severe cardiovascular disease. Ochronosis and alkaptonuria were considered synonymous terms at one time, but it is now agreed that alkaptonuria is only a symptom of ochronosis. So-called exogenous ochronosis, caused by prolonged use of phenol compounds, is not considered here.

Since Virchow's brief description[2] in 1866 of the puzzling condition he called ochronosis, a number of cases have been reported in the literature. Most of these reports have dealt with the clinical significance of alkaptonuria or the clinical picture of the fully developed disease,[3-6] but there are few reports of necropsy cases. Galdston, Steele, and Dobriner,[7] in 1952, reported three autopsied cases of ochronosis, with extensive metabolic studies in two. Lichtenstein and Kaplan,[8] in 1954, discussed the etiology of the disease and pointed out the gross and microscopic features in two autopsied cases. Most of the autopsied cases reported in the literature had severe arteriosclerotic changes in the heart with pigmentation of the valves and, to a less degree, the aorta and coronary arteries. Most reports mentioned briefly the presence of ochronotic pigment in renal tubular epithelium and lumens, although uremia, secondary to ochronotic nephrosis, was not considered the primary cause of death. Severe arteriosclerosis with sequelae, such as myocardial infarction or cerebral softening, was listed as the primary cause of death in most instances.

We are reporting a case of ochronosis in which severe acute renal failure was the immediate cause of death. Vascular disease, although present, played only a minimal part in the symptomatology. Additional data on ochronotic pigment are also reported.

Report of Case

Clinical Course

A 48-year-old woman school teacher was acutely ill, confused, and cyanotic when admitted to the Presbyterian Hospital, at 12:15 a. m. Nausea, vomiting, dyspnea, and fever had developed 24 hours previously and had progressed rapidly. The family stated that, although she had had slight progressive bluish discoloration of the face in the previous year, they had noted rapid development of blue-gray facial discoloration with the onset of her dyspnea. Her blood pressure was 160/90. The respirations were 28 per minute, and the rectal temperature was 105 F. There were scattered moist rales over the chest, and her reflexes

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were hypoactive. Despite oxygen therapy and other supportive measures, there was no change in the "cyanosis," and she died five hours after admission. Adequate laboratory and metabolic studies could not be carried out because of her condition.

After the diagnosis of ochronosis had been established at autopsy, contact was made with the family physician and several members of the family for a detailed past history. The mother and father were second cousins and had 13 children. The remaining 12 siblings were in good health at the time of the report, except for an older sister with mild diabetes.

As an infant the patient's urine was dark and stained the diapers excessively. As an adult, perspiration caused similar staining. During the last 10 years of her life she had frequent positive urine reduction tests for sugar, but blood sugar values were always within normal limits. She had lumbosacral pain that was aggravated by stooping and bending and was not relieved with the use of a sacroiliac brace. She had been hospitalized seven years previously for albuminuria and hypertension. Her blood pressure then was 180/90. Although retrograde pyelograms at that time were within normal limits, there were delayed appearance and poor concentration in the urine of intravenous indigo carmine. Roentgenograms of the spine at that time revealed moderate arthritic changes.

Fig. 1.—Breast plate showing ragged black costal cartilage.

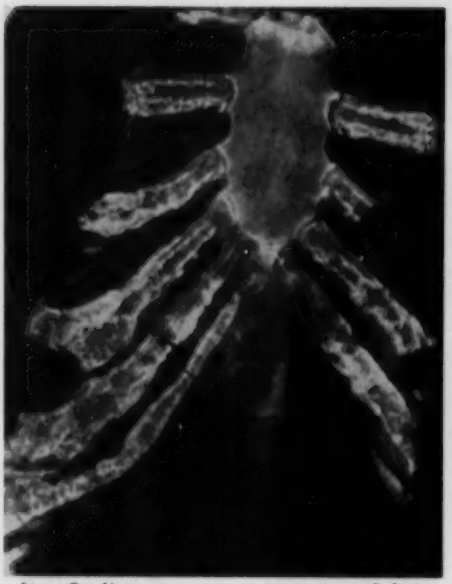
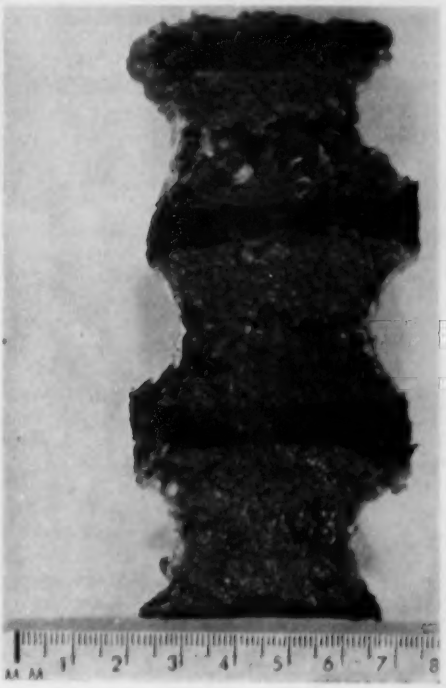


Fig. 2.—Postmortem roentgenogram of breast plate showing focal calcification of costal cartilage.

Fig. 3.—Portion of vertebral column showing thin, focally calcified, black intervertebral disks.



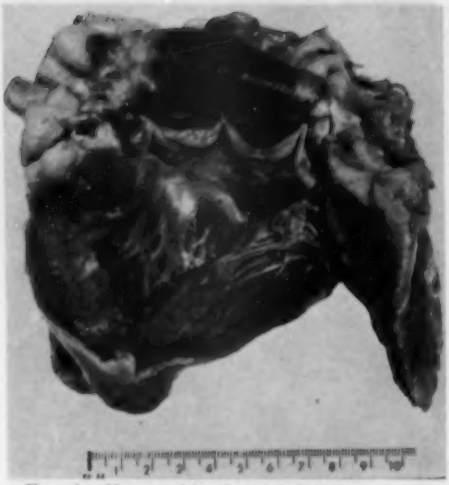


Fig. 4.—Heart with pigment deposits on aorta, aortic and mitral valves, and endocardium, especially at base of papillary muscles.

Autopsy

The body weighed 170 lb. (77.1 kg.) and measured 165 cm. The skin of the entire body, especially that of the face and anterior chest, was bluish gray. There was marked bluish-gray pigmentation of the cartilage of the ears, the bridge of the nose, and the interphalangeal joints. Small brown pigmented spots were present on the cornea. The costal cartilages were black, easily splintered, and focally calcified (Figs. 1 and 2). The periosteum of the ribs, pelvic bones, and skull and the fascia of the anterior chest wall were blue-gray. Especially striking were the jet-black, easily splintered, thin, and hard intervertebral disks (Fig. 3) and the pigmented suture lines of the skull.

The lungs were slightly edematous and contained focal areas of early bronchopneumonia. The cartilaginous rings of the tracheobronchial tree were dark-blue or black and the bronchial mucosa dirty gray. The pulmonary arteries contained several noncalcified gray plaques.

The heart weighed 350 gm. The myocardium was not remarkable. The mitral and aortic valves were thickened and firm and contained numerous blue-gray to black calcified plaques (Fig. 4). The annulus of the mitral valve was calcified, and there was moderate fixation of the leaflets, but the chordae tendineae were unaltered. The orifices of the coronary arteries were surrounded by heaped-up, calcified black plaques. Several similar plaques were present in the coronary arteries, but these did not appreciably narrow the lumen. Black, calcified, and ulcerated plaques were present in the aorta, especially in the abdominal portion. The intima of the aorta was gray, and the media contained focal areas of black pigment. Similar changes were seen in all of the major arteries except those of the brain. The changes were especially prominent in the renal, iliac, and femoral arteries. The intima of the veins was dark gray.

Each kidney weighed 110 gm. Both were coarsely granular and gray-black with narrowing of the cortex (Fig. 5). The corticomedullary junction was indistinct, with the cortex measuring 3 mm. and the medullary portion 8 mm. The right kidney contained a 2 cm. cyst filled with clear fluid and lined with hard black material. The adrenals were small, each weighing 4.5 gm., and the lipid material was depleted. The thyroid was enlarged, weighing 48 gm., and it contained bulging, glistening, tan to dark-red nodules and focal areas of calcium and black pigment.

Microscopic Study

Microscopic changes were most marked in the cartilages, kidneys, and blood vessels. The cartilages contained large amounts of pigment. Dense deposits of pigment were present in the perichondrium. Pigment granules were prominent in the cell capsules and territorial matrix. Frequently the deposits in the latter area were so dense that

Fig. 5.—Kidney showing granular cortex with focal distribution of black pigment.

halos of yellow-brown pigment were present about cell clusters. Pigment deposits were patchy throughout the central portion of the interterritorial matrix. The intervertebral disk substance was fragmented and contained large deposits of pigment that stained yellow, brown, or black, depending on its concentration. The periosteum and surrounding connective tissue were pigmented. Bony trabeculae had focal areas of brown pigment. The bone marrow contained many macrophages with brown pigment granules in the cytoplasm. Similar macrophages

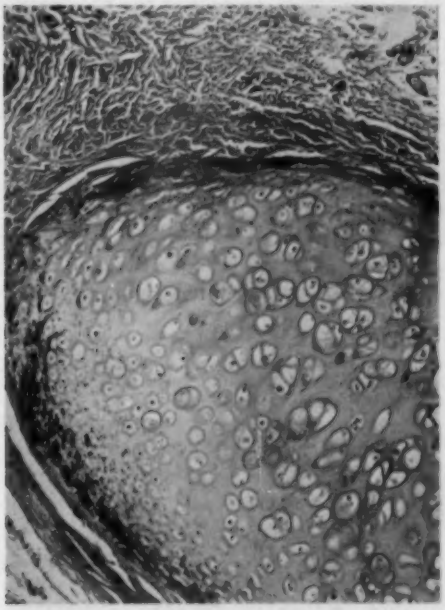


Fig. 6.—Tracheal cartilaginous ring showing distribution of pigment. Hematoxylin and eosin; × 90.

were not found in lymph nodes, liver, or spleen. This pigment did not take the iron stain, but with Fontana and Gomori silver stains the granules were black. Synovium was not examined. The epithelial cells of the mucous glands in the lamina propria of the bronchi contained small masses of pigment (Fig. 7).

Kidney changes were marked. The tubules, especially the proximal convoluted portions, had swollen, necrotic epithelium and, frequently, ruptured basement mem-

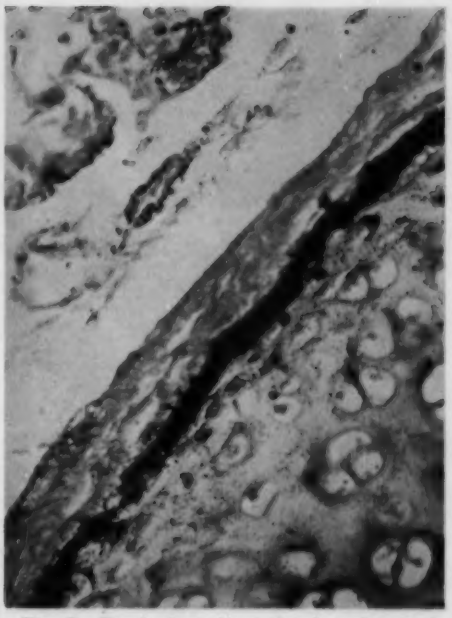
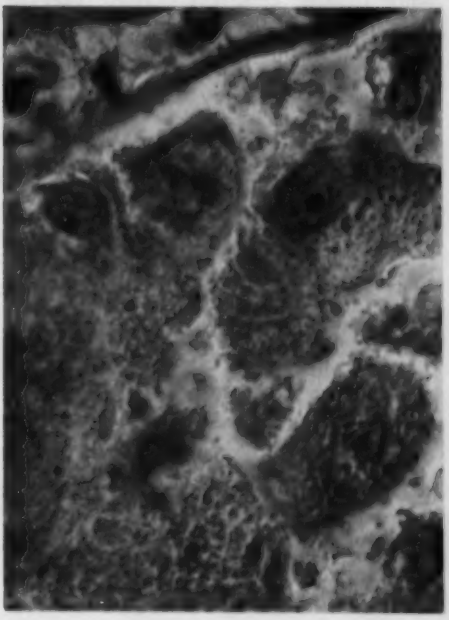


Fig. 7.—Tracheal cartilage showing pigment in perichondrium, cartilage, and epithelium of mucous glands in lamina propria. Fontana silver stain; × 400.

Fig. 8.—Pigment granules in swollen, desquamated cells of proximal convoluted tubules. Hematoxylin and eosin; × 1500.



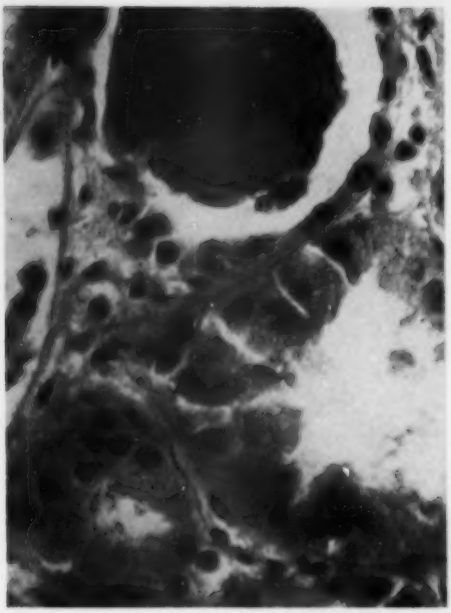
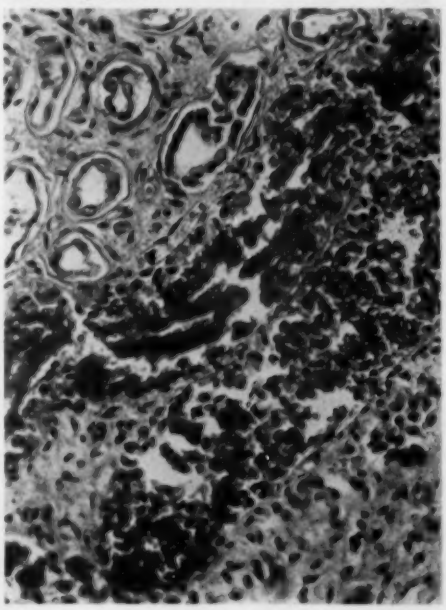


Fig. 9.—Tubule filled with ochronotic pigment. A few pigment granules are seen in cells of adjacent proximal convoluted tubule. Hematoxylin and eosin; × 600.

branes. The epithelium often contained nonrefractile granular pigment (Fig. 8). Many tubular lumens contained varying-sized brown to black pigment casts (Fig. 9). The pigment was found in all portions of the nephron and in many collecting tubules. Many tubules also contained red blood cells (Fig. 10), and many were completely filled with blood. In some areas the red cells were arranged in irregular masses or clumps, sometimes mixed with pigment, and they resembled the "red-cell" or "hemoglobin" casts seen in the kidneys after transfusion reactions. Many of these red cells stained more darkly than usual and were difficult to distinguish from large pigment granules. The possibility was considered that they had in some way been coated with pigment, but this could not be proved. The cyst in the right kidney contained large masses of pigment that stained brown to black with hematoxylin and eosin. Definite ochronotic pigment was not observed in hyalinized glomeruli, although pigment was found in scarred areas outside the glomeruli. There was marked intimal proliferation of arterioles and small arteries, some being almost obliterated. Glomerular changes were compatible with the severity of the arteriolar disease present.

Irregular masses of black pigment were found frequently in the intima and media and occasionally in the adventitia of large arteries. The elastic lamina of many arteries was fragmented and the muscularis interrupted, especially in the areas where pigment deposition was heavy. Large clumps of pigment were present at the base of the mitral valve and in areas of fibrosis of the enlarged, nodular thyroid gland. Yellow-brown granular pigment was present in heptic cells and in focal areas of fibrosis in the myocardium. In many areas, especially adrenal, pancreas, and pituitary, ochronotic pigment deposition was questionable, as differentiation of the ochronotic pigment from pigment normally present and from formalin pigment was difficult. Special

Fig. 10.—Collections of red blood cells and pigment granules in collecting tubules. Hematoxylin and eosin; × 400.



stains, described under "Special Postmortem Studies," were helpful in this respect.

Special Postmortem Studies

Special histologic and chemical postmortem studies were performed. The histologic studies included special stains for demonstration of the ochronotic pigment in various locations, electron microscopy, and special stains for comparison of the ochronotic pigment with melanin of normal white skin and melanin of a heavily pigmented nevus. The chemical studies included extraction and isolation of the ochronotic pigment from cartilage, liver, and kidney.

Histologic Studies.—Methods: All sections were stained with hematoxylin and eosin and by the Nile blue techniques for differentiation of melanin and lipofuscins, as described by Lillie.[9] Where pigment was found by hematoxylin and eosin or the Nile blue stains, the following techniques were also employed: iron stains, Fontana's and Gomori's silver stains, Masson's trichrome stain, the periodic acid-Schiff (PAS) reaction, cresyl violet stain, polychrome methylene blue stain, and Stein's bile stain. Controls included formalin-fixed, paraffin-embedded sections of kidneys from cases of cholemic nephrosis and severe transfusion reactions, normal human white skin, and a pigmented nevus. Sections of the normal skin, the pigmented nevus, and the heavily pigmented cyst in the right kidney of this case also were bleached with hydrogen peroxide and observed by electron microscopy.*

Results: The special stains, with the exception of the Nile blue stains and the silver stains, were not especially helpful, although many showed the pigment quite clearly, in contrast with the tissue background. The Nile blue stain, variant 1, was especially useful as a screening technique, because it showed pigment deposition with little background staining. The Nile blue stain also permitted differentiation of the ochronotic pigment from formalin pigment and from lipofuscin. It did not appear helpful in distinguishing hemosiderin or bile pigment from the ochronotic pigment, as it also stained hemosiderin and bile in control sections. Iron stains of the ochronotic pigment were negative in all locations and positive or negative, where expected, in the controls. The pigment was argyrophilic, but in many areas there was so much silver-positive material or artifact that this method was not useful for screening slides for pigment deposition.

After the special stains were studied, the pigment deposits in the adrenal were regarded as those normally present rather than as ochronotic pigment, and the small amount of pigment present in the islet tissue of the pancreas was regarded as artifact from formalin fixation. Small collections of pigment in the fibrous-tissue trabeculae of the anterior pituitary were regarded as ochronotic pigment. The normal pigment of the posterior pituitary was clearly outlined by the Nile blue stains.

During early comparison of the ochronotic pigment with melanin pigment of normal white skin and melanin of a pigmented nevus, it was thought that certain differences between ochronotic pigment and melanin were observed in the trichrome, cresyl violet, and PAS stains, but further studies indicated that these variations were dependent only on differences in amount of pigment present. Ochronotic pigment and melanin of normal skin and of a pigmented nevus were positive to the Nile blue stains and were bleached slightly with hydrogen peroxide. No differences in either the extracellular or the intracellular melanin and ochronotic pigments were observed by electron microscopy.

Chemical Studies.—Methods: A nonprotein nitrogen determination was made on hemolyzed blood obtained at autopsy, and qualitative tests for homogentisic acid were performed on 5 cc. of dark-amber urine obtained from the bladder. Attempts were

* The electron microscopy was performed on a Phillips E-100 instrument at magnifications up to 32,000 ×, through the courtesy of Dr. J. Wolken, of the Biophysics Research Laboratory of the Eye and Ear Hospital of Pittsburgh.

made to dissolve the pigment from the cartilage in organic solvents, sulfuric acid, sodium hydroxide, and hydrogen peroxide.

Isolation of the pigment from the cartilage, liver, and kidney was performed by the method of Janney.[10] Using this technique, 19.7 gm. of costal cartilage was boiled and dissolved in concentrated hydrochloric acid, after which the solution was neutralized with sodium carbonate and the pigment precipitated by adding an equal volume of 25% acetic acid and allowing it to stand overnight. The precipitate was then thoroughly washed with 12.5% acetic acid, dissolved in 10% sodium carbonate, and precipitated a second time with an equal volume of 25% acetic acid. This second precipitate was first washed with 12.5% acetic acid, then with water, and dried at 37 C. After drying, the precipitate was extracted with alcohol, followed by ether. A small amount of additional pigment was obtained from this same cartilage by filtering the washings from the first precipitate and by processing the residue as above. Liver and kidney tissue were also utilized, although additional precipitations, filterings, and centrifugings were necessary because of the tendency of these lighter, floating tissues to entrap the pigment granules.

Results: The blood NPN was 294 mg/100 ml. Qualitative tests for urinary homogentisic acid were negative. The pigment in the cartilage was insoluble in organic solvents and concentrated sulfuric acid. It was quickly, but not completely, soluble in sodium hydroxide. It was slightly dissolved in hydrogen peroxide after 24 hours.

The amount of pigment isolated from cartilage was 216 mg. from 19.7 gm. (approximately 1.1 gm/100 gm.). Two milligrams of pigment was extracted from 86.7 gm. of liver tissue (2.3 mg/100 gm.), and 1 mg. was obtained from 31.2 gm. of kidney tissue (3.3 mg/100 gm.). Control experiments with normal formalin-fixed kidney and liver tissue gave neither quantitative nor qualitative indications of ochronotic pigment.

Comment

Ochronotic pigment in the kidneys has been mentioned in previous reports of autopsied cases, but other pathologic changes were usually considered more important than the renal disease as the primary cause of death. In the case reported here the patient was considered to be in reasonably good health, but she died of severe renal damage approximately 72 hours after the onset of acute symptoms.

The probable explanation of the pigment-containing macrophages found in large numbers in the bone marrow, but not in the rest of the reticuloendothelial system, is that the pigment had been released from the damaged, often fragmented bone in the areas of osteoarthritis and then picked up by the macrophages.

The exact nature and chemical composition of the pigment in ochronotic subjects have not been established, although it has been accepted as a melanin or melanin-like substance. Our preliminary studies by special staining and electron microscopy showed no differences between ochronotic pigment and melanin. Further studies of ochronotic pigment by available chemical and physical methods are in progress.

Summary

A case of hereditary ochronosis in a 48-year-old woman with death from ochronotic nephrosis is reported. The characteristic features of dark urine, osteoarthritis, and pigmentation of cartilage were present in this case. No differences between ochronotic pigment and melanin obtained from a pigmented nevus could be demonstrated by electron microscopy or special stains. Of the special stains employed, Lillie's Nile blue method was most useful in screening sections for presence of pigment and for differentiation of ochronotic pigment and melanin from formalin pigment and lipofuscins. Extraction and isolation of the

ochronotic pigment indicated concentrations of approximately 1.1% in cartilage, 0.002% in liver, and 0.003% in kidney.

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Sex Differences in Incidence and Severity of Lymph Node Lipogranulomatosis

A Similarity to Atherosclerosis

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It has been observed that lipid deposits with varying degrees of cellular reaction may be present in lymph nodes that are in the region of excessive tissue lipid release. These lesions may at times progress to the point of being histologically indistinguishable from the sarcoid granuloma. Such lesions have been noted in regional lymph nodes in areas of tumor, lipid pneumonia, hematoma, and cholelithiasis, as well as numerous other conditions. This reaction in the lymph node has been designated as a lipogranuloma. It seems rather obvious that the lymph nodes located at the junction of the cystic and hepatic ducts would be a common site for this lipogranulomatous reaction because of disturbances in local lipid transport associated with cholecystitis and cholelithiasis. One observer [1] reported 22 instances of lipogranuloma in 31 lymph nodes removed along with cholecystectomy. In the present study lipogranulomatous reaction in such lymph nodes that drained the areas of cholecystitis and cholelithiasis was found almost as frequently. In the occidental world there are sex differences in the incidence and severity of coronary atherosclerosis. It is generally estimated that in the absence of diabetes or hypertension sudden death from coronary atherosclerosis is 10 to 20 times more frequent in men under the age of 50 than in the women.[2] Whatever are the basic pathogenetic mechanisms of atherogenesis, one fact remains certain, and that is that the lipid deposit and the reaction thereto are an important part of the atherosclerotic lesion. Therefore it seemed that it would be of interest to determine whether sex differences existed in the ability of tissue, other than vascular, to handle excessive amounts of extraneous lipid. These regional lymph nodes associated with chronic cholelithiasis would appear to afford this opportunity. Furthermore, such tissue would not be subject to the mechanical factors of the hemodynamics of the circulation or the peculiarities of the structure of various arterial walls.

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From the Department of Laboratories, Beth-El Hospital.

Dr. Benjamin Kogut furnished a number of lymph nodes secured during surgery.

This study was aided by a grant from the Max and Helen G. Borgenicht Foundation, Inc.

Material

One hundred twenty-five consecutive cases of chronic cholecystitis and cholelithiasis in which regional lymph nodes were removed were studied. Of these patients, 81 were female and 44 were male. This, however, did not reflect the true incidence of gallbladder disease in males as seen at this institution because a greater effort was made to obtain the lymph nodes in the males in order to increase their relative number for the purposes of this study. One or more lymph nodes in each case were resected from the region of the cystic duct. Sections were made of the gallbladder walls and lymph nodes and were stained with hematoxylin and eosin. At the beginning of this study the lymph nodes were also subjected to frozen section and stained with Sudan III. On several occasions fresh lymph node tissue was studied under polarized light. A number of nodes were stained by means of the Schiff-periodic acid technique. The degree of lipogranulomatosis was arbitrarily graded as none present, minimal, moderate, and marked. These sections were studied as coded slides without knowledge of the sex or age in each particular case. In all instances cholelithiasis was

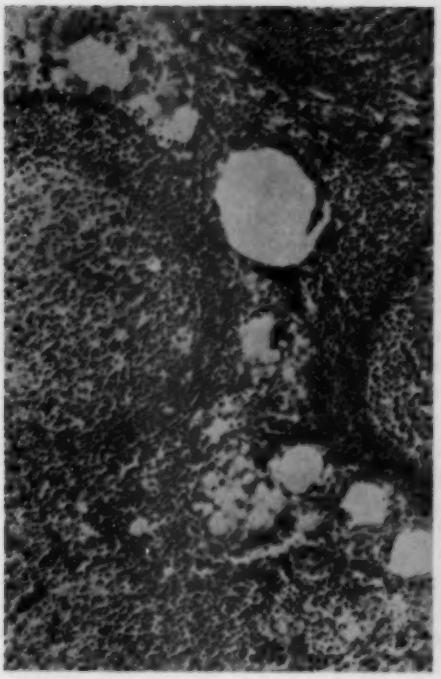

Fig. 1.—Lymph node with varying-sized extracellular lipid vacuoles, some of which are coalescing.

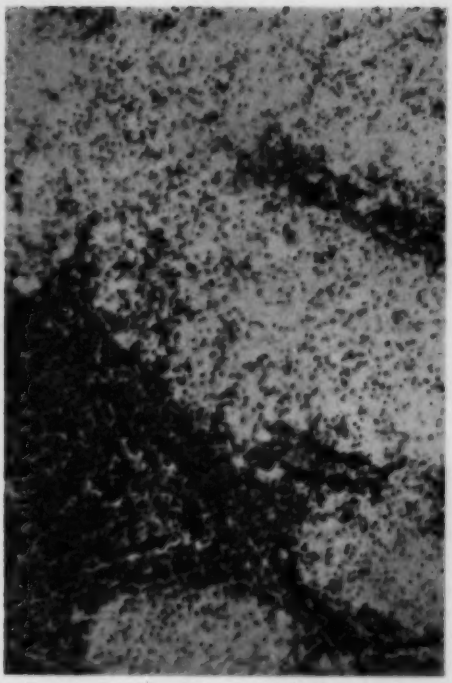

Fig. 3.—Lymph node with epithelioid cell type of reaction in an area of lipid infiltration.

Fig. 2.—Lymph node with intra- and extracellular lipid vacuoles with giant-cell reaction.

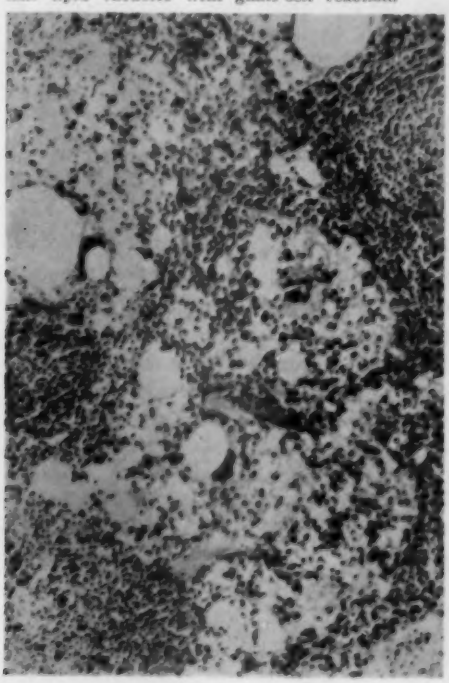

Fig. 4.—Lymph node with characteristic granulomatous giant cell containing tubercle in an area of lipid infiltration.

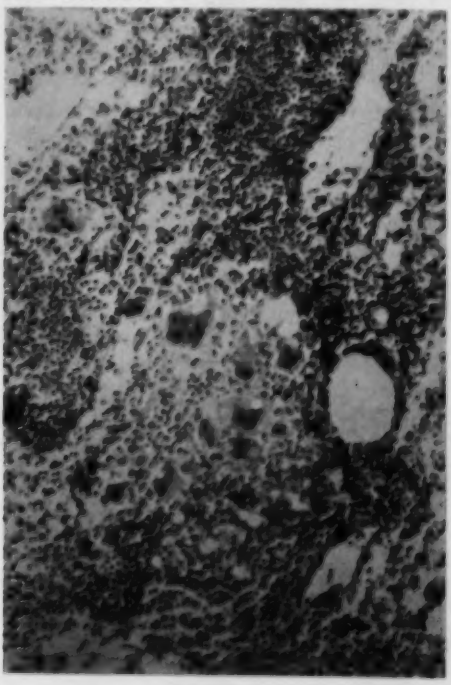

TABLE 1.—*Extent of Lipogranulomatous Involvement of Lymph Nodes in Women*

Age	Total No. Cases	Involvement				Total No. with Lipogranulomatosis
		None	Minimal	Moderate	Marked	
Under 46	40	29	6	4	1	11 (27%)
Above 45	41	17	10	9	5	24 (58%)
Total	81	46	16	13	6	35 (44%)

present and was associated with various degrees of cholecystitis. Unfortunately, chemical analysis of the calculi was not done.

In addition, the lymph nodes at the neck of the gallbladder were removed and similarly studied in 25 autopsies on adults who had died suddenly from trauma and in whom the gallbladders were anatomically without significant change or calculi. In these lymph nodes there was no evidence of any lipogranulomatous lesions. A few scattered extracellular vacuoles were seen in two of these lymph nodes.

The lesions in the lymph nodes from the cases of cholelithiasis varied in appearance with the degree of lipid deposition and extent of the cellular reaction. In the least involved cases lipid was found in the form of varying-sized extracellular, as well as intracellular, small vacuoles. As the lesions become more advanced and extensive, numerous vacuolated lipid-containing macrophages were present, and the smaller extracellular lipid vacuoles coalesced into larger ones. These were often surrounded by macrophages and giant cells. Many of these progressed into the formation of epithelioid tubercules, some of which became fibrotic (Figs. 1, 2, 3, and 4). As the lesions progressed in age, less lipid was noted in them. Frozen sections stained with Sudan III were positive in all instances in which these lesions were present. The periodic acid-Schiff reaction revealed only on occasion a scant amount[8] of periodic acid-positive material. Under polarized light the fresh tissue revealed Maltese cross configurations.

Findings

No relation was noted between the degree of lipogranulomatosis in the lymph nodes and the extent of the inflammatory or fibrotic process in the gallbladder walls. Since cholelithiasis was the indication for cholecystectomy in all these cases, this was a constant factor. There was also no relationship between the amount of lipid in the lymph nodes and the occurrence of cholesterosis in the gallbladder wall.

The incidence and degree of lipogranulomatous lymph node involvement and its relationship to age and sex may be noted in Tables 1 and 2. In a total of 125 cases, 84 had some degree of lipid in the lymph nodes. This involvement was twice as frequent in the men as in the women. Under the age of 46 lymph-node lipogranuloma was present four times as frequently in men than in women. The severity of involvement paralleled this difference in sex incidence, so that 20 of 44 men had marked lipogranulomatosis, whereas only 6 of 81 women showed a similar advanced degree of involvement. The differences between women below the age of 46 and those above that age were also quite striking. Women past the age of 46 had twice the frequency of involvement. In this group 41 showed marked to moderate involvement, whereas only 5 out of 40 women less than 46 showed moderate to marked involvement.

TABLE 2.—*Extent of Lipogranulomatous Involvement of Lymph Nodes in Men*

Age	Total No. Cases	Involvement				Total No. with Lipogranulomatosis
		None	Minimal	Moderate	Marked	
Under 46	13	2	1	7	3	11 (84%)
Above 45	31	3	5	6	17	28 (90%)
Total	44	5	6	13	20	39 (88%)

Comment

The lymph nodes at the junction of the cystic and hepatic ducts in many cases of gallbladder disease are the frequent recipients of various amounts of lipids secondary to derangement of lipid transport in the gallbladder. The lymph node in itself is not involved in the primary disease process. Within the lymph node much of this lipid is engulfed by macrophages, and a cellular reaction is evoked in response to it, just as a cellular reaction is invoked in response to lipids in arterial walls. The role of estrogens in the pathogenesis of atherosclerosis has been demonstrated both in experimental animals and in humans. Estrogens will inhibit the development of experimental atherosclerosis in chickens.[3] Oophorectomized women show more atherosclerosis than other women of a comparable age group.[4] Castrated men show less atherosclerosis than normal men of a comparable age group.[5] Atherosclerosis in the nonhypertensive and nondiabetic woman usually becomes a problem only in the postmenopausal period. Most of this sex difference has been related to the systemic effect of hormones, in particular estrogens, upon lipid transport and metabolism. Whether or not these hormonal factors that account for sex differences in degree of atherosclerosis operate on a local tissue level has never been demonstrated. In this study the incidence and degree of lymph node lipogranulomatosis parallel the sex and age differences that are seen in coronary atherosclerosis. These differences cannot be accounted for simply on a basis of differences in duration of the gallbladder disease, since the younger males revealed more involvement than the older females. Nor can it be accounted for in the differences in the intensity of gallbladder disease, since there was no relationship to this. Therefore the findings in this study are particularly suggestive of the fact that hormonal factors associated with sex differences may not only effect lipid metabolism systemically but also influence the local tissue transport and cellular response to excessive extraneous lipids. This difference in the degree of lipogranulomatosis in the lymph node obviously is not related to the hemodynamics of the circulation or differences in structure of different arterial walls. Experimental studies regarding this concept are in progress.

Summary

1. The lymph nodes at the junction of the hepatic and the cystic duct revealed lipogranulomatous lesions of varying degrees of intensity in 84 out of 125 cases of cholelithiasis.

2. These lesions varied in incidence and severity according to sex and age, with the men showing a preponderance of lesions, and were similar in this respect to the sex and age variation seen in coronary atherosclerosis in white persons in this area of the world.

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Glomerular Alterations in Kidneys of Rats Treated with Desoxycorticosterone

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In a series of investigations on the effect of various adrenocortical hormones upon the blood vessels of the eye and kidney of experimental animals, it was noted that DCA (desoxycorticosterone acetate) produced marked alterations in the glomeruli of the rat. The glomerular alterations were more evident when the tissue was examined with ultraviolet light than when studied following special staining procedures.

Selye,[1] in 1943, carried out experiments with heminephrectomized rats which were given DCA. Severe vascular lesions were produced in these animals, particularly in the kidney, and the animals became hypertensive. The present observations were made concomitantly with another project designed for study of glomerular changes in kidneys of patients with essential hypertension, "collagen" disease, and nephritides. The renal stromal changes induced in rats by DCA had special properties not found so far in any natural disease state investigated.

Materials and Methods

Rats of the Long Evans strain, usually males weighing about 350 gm., were given subcutaneous injections of 1 mg. of DCA daily for 42 days. Isotonic saline was given as drinking fluid to half of this group. In addition, a group of diabetic rats having blood sugar levels ranging between 375 and 516 mg. per 100 cc. were given DCA for 42 days. Some of these animals were given isotonic saline to drink. Suitable untreated controls were used. Animals were killed by exsanguination after being anesthetized with ether.

All kidney tissues were fixed in 10% neutral formalin and embedded in paraffin. Sections were prepared with allochrome, periodic acid-Schiff (PAS), Mallory, and hematoxylin-and-eosin stains.

For the ultraviolet microscopy paraffin sections 4μ or less in thickness were deparaffinized with xylene and mounted in glycerol. Vycor slides and cover slips were used. Design, technical aspects, and use of the Polaroid color-translating ultraviolet microscope employed in this study are described elsewhere.[2,3]

Three sets of ultraviolet wavelengths were used for the study of each glomerulus (Table 1).

Results

The ultraviolet absorptions of the capillary stroma of glomeruli were altered in all animals treated with DCA. In the three sets of wave lengths used, renal stroma of untreated control rats appeared color-translated as red-violet, gray-tan, and light tan in Sets 1, 2, and 3, respectively (Fig. 1 shows Set 3). Following administration of DCA, however, Set 1 was unchanged; Set 2 was translated as gray-brown, and Set 3, as dark brown (Fig. 2 shows Set 3). Comparisons of kidneys from normal animals and from animals treated with DCA are shown in Table 2. As indicated, the most

TABLE 1.—*Ultraviolet Wavelengths Used in Study*

	Blue, Mμ	Green, Mμ	Red, Mμ
Set 1	280	265	240
Set 2	280	253	248
Set 3	248	240	235

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From the Department of Anatomy, State University of Iowa College of Medicine; the Departments of Pathology, Massachusetts Memorial Hospitals and Boston University School of Medicine, and the Cancer Research Institute, New England Deaconess Hospital, Boston.

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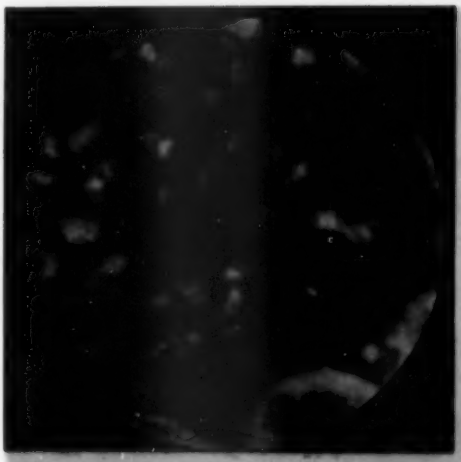


Fig. 1. — Portion of a glomerulus from a normal rat kidney. Third set of wave lengths, 248μ, 240μ and 235μ. The intercapillary stroma is light tan and the red blood cells pinkish. Ultraviolet color-translated photomicrograph; × 500.

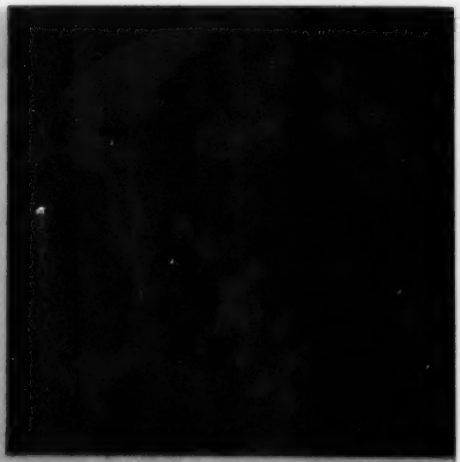


Fig. 2. — Portion of a glomerulus of a diabetic rat treated with DCA for 42 days. Wave lengths same as in Figure 1. The intercapillary stroma is dark-brown and the red blood cells are yellowish pink. Ultraviolet color-translated photomicrograph; × 500.

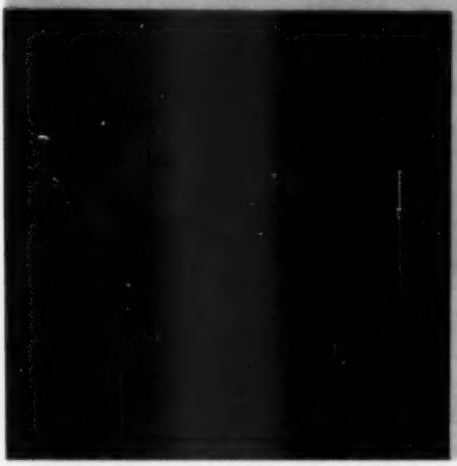


Fig. 3. — Portion of a glomerulus of a diabetic rat treated with DCA and given isotonic saline as drinking water for 42 days. First set of wave lengths 280μ, 263μ, and 240μ. Note erythrocyte rouleaux in capillaries. Intercapillary stroma is red-violet. Ultraviolet color-translated photomicrograph; × 500.

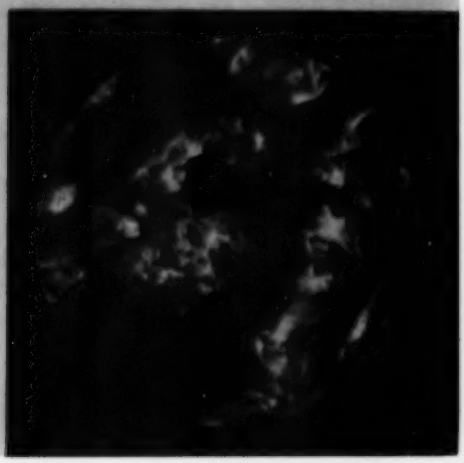


Fig. 4. — Longitudinal section through a small artery of rat kidney. Animal was treated the same as in Figure 3, set 2. Individual erythrocytes and clumps of red blood cells are visible. The stringy fibrin-like material, of yellow-tan color, is clearly shown between and around the erythrocytes. Ultraviolet color-translated photomicrograph; × 500.

TABLE 2.—*Color-Translated Ultraviolet Absorptions of Rat Renal Glomerular Stroma*

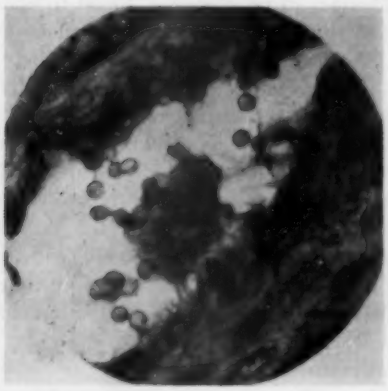
Rat Group	Set 1	Set 2	Set 3
Normal	Red-violet	Gray-tan	Light tan
DCA	Red-violet	Gray-brown	Dark brown
Diabetic	Red-violet	Orange-brown	Orange
DCA-diabetic	Red-violet	Gray-brown	Dark brown

marked difference is seen in the shortest ultraviolet wavelength range.

Glomeruli from untreated diabetic rats had a different ultraviolet absorption pattern than was found in intact normal animals. In the diabetic rat the color of the intercapillary stroma was translated as orange in Set 3, while it was light tan in the normal rats (Table 2). Similar observations have been made on the human diabetic.[4] When the diabetic rat was treated with DCA for 42 days, however, the orange was completely masked by the dark-brown color, which is characteristic for the DCA-induced change (Fig. 2).

Since the ultraviolet absorptions of the glomerular stroma in DCA-treated rats were different from anything seen previously in this animal, the material was compared with observations which had been made on kidneys of human beings with a variety of diseases. Early studies[4] had shown that in the glomerular stroma ultraviolet absorptive characteristics were different in cases of essential hypertension, pyelonephritis, diabetic nephropathy, glomerulonephritis, amyloidosis, lupus erythematosus, and periarteritis nodosa. The changes after DCA in the rat closely resembled the ultraviolet absorption found in cases of amyloidosis.

Fig. 5.—From same animal illustrated in Figure 4. Picture taken at 240 mμ. The clumping of the red blood cells in the artery is shown clearly, and the intravascular fibrin-like material is evident along the endothelium and between erythrocytes; × 500.



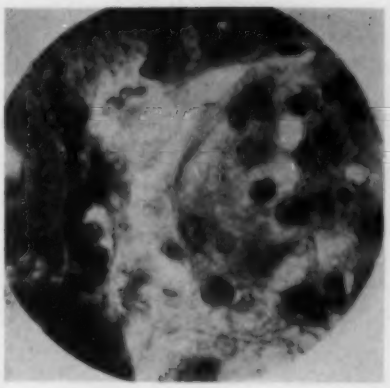


Fig. 6.—Glomerular capillaries on the right and venule on the left. Both are lined by adherent stringy material strongly absorbed at 240 mμ after treatment with DCA and saline; × 500.

An additional alteration not encountered before was seen in the glomeruli of DCA-treated rats when examined by ultraviolet microscopy. A stringy material was found lining the endothelium of all glomerular capillaries. This adhesive coating absorbed in colors translated as red-violet in Set 1 and bright rose-pink in Sets 2 and 3 (Fig. 3 for color, Fig. 6 for black and white). Since it was suspected that this coating was a fibrin-like material, rat fibrin, from either whipped or clotted blood, was examined with ultraviolet light. Fibrin showed the same absorption pattern as the material lining the glomerular capillaries. After DCA treatment, the erythrocytes in glomerular capillaries, as well as in liver sinusoids and veins, were frequently in rouleau formation and were usually coated with the fibrin-like material. A photograph of such erythrocytes in a glomerulus is shown in Figure 3. Also, clumping of red cells was seen in the arteries of the kidney (Figs. 4 and 5).

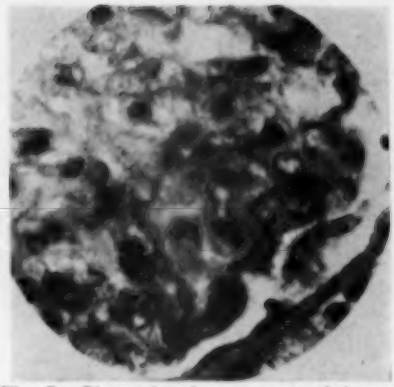
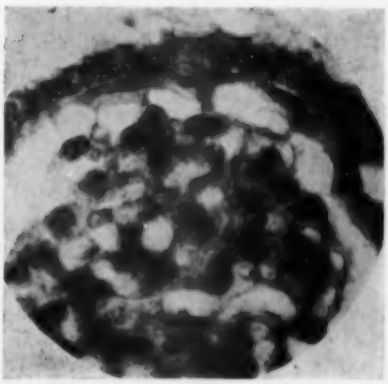


Fig. 7.—Glomerulus from a necropsied case of toxemia of pregnancy. Picture taken at 240 mμ. Capillaries are lined with strongly absorbing material, which is black in the print; × 500.

Because the coating substance seemed most likely to be fibrin, tissues were examined from other conditions in which fibrin formation is known to be important. Dr. Donald G. McKay, Boston Lying-in Hospital, made available kidneys from two cases of human toxemia of pregnancy and several kidneys from rabbits with the generalized Shwartzman reaction. Examination with ultraviolet microscopy of the kidneys from these sources showed both the same type of stringy protein-like material and erythrocyte adherence. The above conditions have been ascribed to abnormal fibrin precipitation,[5,6] and in conventionally stained preparations of these kidneys the material appeared to be fibrin (Figs. 7 and 8). The ultraviolet absorption characteristics of this material were the same as seen in the lining of capillaries after DCA treatment. The ultraviolet absorption of the glomerular stroma in the cases of toxemia of pregnancy and animals with Shwartzman reactions, however, appeared to be normal. An apparent hypercoagulability of blood and an excessive fibrin deposition in glomerular capillaries was shared by these conditions and DCA treatment.

Fig. 8.—Portion of glomerulus from a rabbit with generalized Shwartzman reaction. Picture taken at 240 mμ. The fibrin-like material is shown as black threads in the space of Bowman's capsule and lining capillaries; × 500.



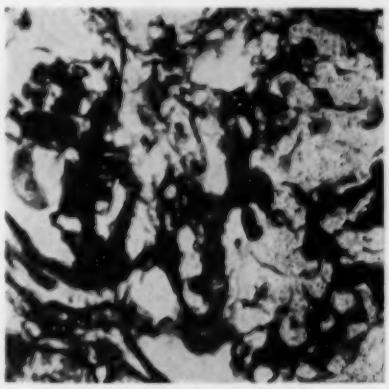


Fig. 9.—Portion of glomerulus from diabetic rat treated with DCA and given physiological saline. The axial stroma is evident, but the histochemical peculiarities of the stroma as seen with ultraviolet light is not seen. Allochrome stain; × 500.

Changes in rat glomeruli found in microscopic sections stained with the allochrome or PAS methods were not so striking. In the rats given DCA and saline, with or without diabetes, some glomeruli showed an irregular thickening of the axial stroma, staining olive-brown with allochrome and being PAS-positive. Erythrocyte rouleau formations were found, but the thin adhesive lining of glomerular capillaries was not visible (Fig. 9). Controls scrutinized with equal care by ultraviolet microscopy and special stains gave no evidence of similar changes.

Comment

Ultraviolet microscopy used as a supplementary technique to histochemistry shows some promise of assisting in the elucidation of problems involving altered kidney structure. In the present project a qualitative alteration in renal glomerular stromal properties was found after animals had been treated with DCA.

Why the abnormal absorptive characteristics of glomerular stroma in DCA-treated rats were like those in amyloidosis is unclear. Constant use of the Polaroid instrument has shown that technical factors, section thickness, fixative, local peculiarities of tissue, and species are not involved. Perhaps the simulation of amyloidosis is a coincidence. If DCA with its known property of increasing vascular permeability had allowed the larger protein molecules in the plasma access to the intercellular space of the intercapillary glomerular stroma, the similarity to the condition found in amyloidosis would perhaps be explained. Recent fluorescent antibody studies have shown that in amyloidosis and glomerulonephritis globulins become localized in glomerular stroma.[7,8] Proof is lacking that this occurred in the present experimental studies.

The identification of a stringy adherent layer lining the glomerular capillaries and coating erythrocytes after DCA could have several interpretations. However, because it had ultraviolet absorption properties identical with rat fibrin and since it showed properties similar to fibrin found in the kidneys of persons with toxemia of pregnancy and animals with the experimental Shwartzman reaction, this material is believed to be fibrin.

With this evidence some additional light may be thrown upon the mechanism whereby renal lesions like those described by Selye [1] may be produced. Following DCA treatment of rats the glomerulus is apparently altered by an increased local accumulation of macromolecules including globulins in vessel walls and adjacent stroma and by deposition of a sticky protein material on the endothelium. If the mechanical force of induced hypertension were added, more extreme permeation of vessel walls by large protein molecules including fibrin would be anticipated, and the lesions of the glomeruli as well as the inflammation of blood vessel walls would be accentuated and be similar to those described by Selye.[1]

Actually the present experiments were carried out on apparently normotensive rats, but the animals were perhaps killed too soon to produce the most marked severe stages of vascular alteration. Hypertension per se could scarcely have caused the glomerular abnormalities described. Human kidney biopsy specimens taken from patients with hypertension who had undergone sympathectomy or material from necropsies of persons who had had essential hypertension showed no such lesion.[9] Furthermore, the combined glomerular stromal changes and the accumulation of the intravascular fibrin-like material observed with ultraviolet light were not present in any other kidney disease investigated.

From these meager experimental data no extensive positive conclusions are possible. However, it seems clear that when DCA was given to rats it produced glomerular changes unlike those seen in any of a variety of naturally occurring kidney diseases. Whatever life stresses may produce these diseases, it does not seem that the special situation achieved with DCA is implicated.

Summary

Rats treated with DCA showed alterations of the renal glomerular ultraviolet absorptions when investigated with the Polaroid color-translating microscope. In glomerular stromal tissue the changes qualitatively closely simulated amyloidosis. Glomerular capillaries and erythrocytes were coated also with a protein-like material thought to be fibrin. Glomerular structural alterations were less apparent with special stains than when examined with ultraviolet light. The experimental findings differ from changes

observed previously in naturally occurring renal diseases in human beings, including essential hypertension and periarteritis nodosa.

Dr. Shields Warren did the studies using the Polaroid microscope; the Office of Naval Research provided the microscope on loan, and Mrs. Kathryn H. Haley gave technical assistance.

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The Tubule Plucking Test for Testicular Atrophy

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Examination of the testis at necropsy should include an attempt to draw out tubules from the cut surface. Failure of this test, in the adult, is generally regarded as an indication of testicular atrophy. The following observations were made in an attempt to elucidate the mechanism and significance of this test.

Materials and Methods

Both testes were examined at necropsy from 81 men over 50 years of age, 12 stillbirths, 4 boys in the first decade of life, and 4 boys in the second decade. Biopsy specimens from one testis of six cryptorchids were also examined.

All necropsy specimens were treated similarly. The testis was first bisected from the convex surface so that the incision extended into the mediastinum. The cut surface of one-half of each testis was gently plucked several times with fine pointed forceps; this test was repeated under the dissecting microscope (magnification, 8.75 diameters). The other halves were fixed in formol-sublimate (Lendrum[5]) for 24 hours; at the end of this time a piece (approximately 2 mm. thick) was taken from the entire width of the specimen. This method gives adequate fixation and prevents distortion of the interstitial tissue, which occurs if the piece for section is taken prior to fixation.

Paraffin sections were stained with Ehrlich's hematoxylin and eosin, Weigert's resorcin-fuchsin method for elastic tissue, and Gordon and Sweets'[4] method for reticulin fibers. Sections were prepared of tubules which had been drawn out from the cut surfaces of testes by laying the tubules across a glass slide coated with Mayer's albumen and fixing in formol-sublimate for three hours. The strands were then held in melted agar, which when cool formed a block that, when embedded in paraffin, could be cut and stained in the usual way.

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From the Department of Pathology, University of Cambridge.

Results

Strands can often be drawn out to a length of 10 cm. or so from the cut surface of a normal adult testis (Fig. 1). Failure to pluck out tubules was noted in the prepubertal testes and in testes showing the histological features of atrophy, to be discussed later. The test should be repeated several times, as failure may occur in a normal testis if a septum or the collagenous stroma of the mediastinum testis is picked up.

Fig. 1.—Strand plucked from the surface of a hemisected normal adult testis.

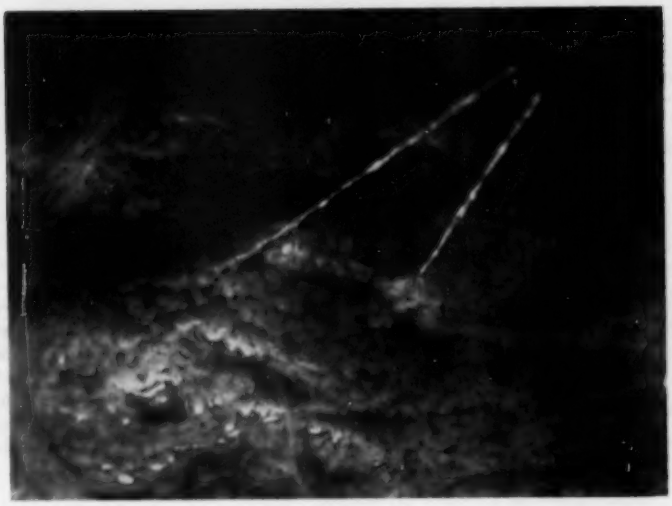


Fig. 2.—Unfixed cut surface of normal adult testis, showing strands sliding out of a "sheath." The coiled seminiferous tubules are clearly visible. × 28.

Under the dissecting microscope the cut surface of the normal adult organ is translucent gray and speckled with brown dots, which are clusters of Leydig cells; the atrophic testis has a dull gray, rather opaque, appearance. Seminiferous tubules appear as gelatinous coils in the normal, and when plucked the strand so produced appears to slide out of a sheath (Fig. 2). Sections of such strands were compared with cross sections of tubules in situ to determine the nature of this sheath. In the normal adult testis the epithelium of the seminiferous tubule is surrounded by a thick, continuous argyrophilic strand, as shown by methods for reticulin, which closely follows the contours of the basal layer of epithelial cells. External to this are a few fragmentary argyrophilic strands embedded in a matrix which stains purple with the periodic acid-Schiff method. This matrix and the argyrophil strands are the basement membrane of the epithelium. Surrounding it is a layer of collagen fibers, in which are a few fibroblast nuclei and two or three strands of elastic tissue in the periphery; the last two layers are the tunica propria. The interstitial tissue of the testis is composed of collagen fibers, much more numerous in the prepubertal than in the mature testis. Interstitial cells are present for the first week or so after birth and gradually disappear over the first few months. The Leydig cells appear near puberty and vary greatly in number and distribution in the normal testis. Elastic tissue is absent from the testis, except in the blood vessels, before puberty (Sniffen[7]); its appearance coincides with the onset of spermatogenesis and a considerable increase in diameter of the seminiferous tubule.

In the biopsy specimens of one testis examined from a cryptorchid elastic tissue was absent from the tunica propria, tubules being considerably smaller in diameter than those of the adult testis (average diameter, 90μ, as opposed to from 150μ to 300μ in the normal). Only in one case was a single strand of elastic present in some seminiferous tubules.

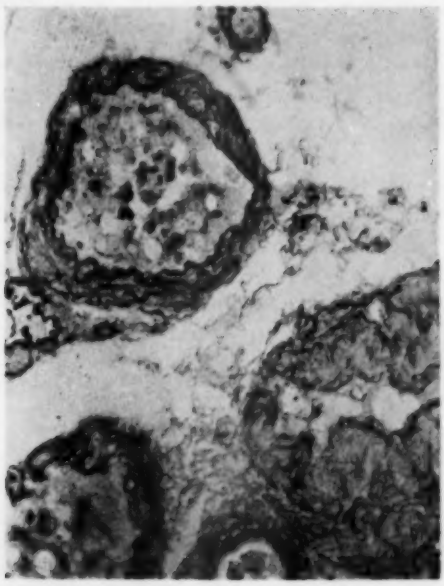
Comparison of sections of plucked "tubules" with the normal showed that the plane of cleavage lay in the collagenous layer of the tunica propria, the plucked "tubule" consisting of epithelium and basement membrane only (Fig. 3); no elastic tissue was demonstrable in the sections prepared.

Of the 81 pairs of testes examined from men over the age of 50, tubules were not plucked out from the cut surface in 29. The epithelium in all of the latter showed

Fig. 3.—Section of strand plucked from the testis. It consists of fragmented epithelium and the basement membrane, with some of the collagenous tunica propria. Hematoxylin-eosin; × 380.

absence of spermatogenesis, and in some all trace of epithelium had disappeared, together with its basement membrane (Fig. 4). In 24 pairs the tubules showed conspicuous collagenous thickening of the inner layer of the tunica propria; in 5 a considerable increase of interstitial collagen, together with collagenous thickening of the tunica, was evident.

Fig. 4.—Atrophic seminiferous tubules, showing absence of basement membrane in one tubule devoid of epithelium. Gordon and Sweets' method; × 380.



Comment

The results suggest that the commonest type of atrophy after the fifth decade is associated with changes in the tunica rather than the interstitium, though this tubular change was also seen in the smaller number, showing interstitial fibrosis. In those cases in which the tunical thickening was barely detectable the epithelium showed a maturation arrest of spermatogenesis, spermatids being scanty; mature spermatozoa were hardly ever seen. The epithelial changes probably precede those in the tunica, though Nelson and Heller [6] consider the changes in the tunica to be the primary abnormality. Whatever the type of atrophy, tubular or interstitial, changes are seen in the collagenous layer of the tunica, which is the plane of cleavage produced in the normal testis by the plucking test. These changes probably account for failure to draw out tubules from the surface of an atrophic testis, and this explanation is opposed to the view, generally held, that fibrosis between the tubules is the main operative factor.

The presence of elastic tissue in the tunica propria seems to be associated with sperma-

togenesis; once found, it persists and is clearly visible in advanced cases of atrophy when appropriately stained. Tubular diameter increases considerably with spermatogenesis; this factor, together with the varying luminal pressure induced by waves of spermatogenesis passing along the tubule (Curtis [3]), may promote its appearance. The appearance of elastic tissue in myocardial scars (Blunting [1]) supports this view. In the testis elastic tissue is probably derived from collagen in the tunica propria; recent electron microscopy and biochemical studies of collagen from abdominal skin (Burton et al.[2]) also favor the hypothesis that elastic tissue is derived from collagen.

Elastic tissue was not found in the tunica propria of the tubules of testes taken from five cryptorchids. However, two of the patients have children, so that, spermatogenesis being normal in the other testis, it is unlikely that the stimulus promoting elastic tissue formation is humoral.

Summary

The "tubule"-plucking test for testicular atrophy is described and its significance discussed.

Studies of the testis provide some evidence of the stimuli promoting formation of elastic tissue.

Dr. A. M. Barrett gave stimulating advice and encouragement, and my colleagues Drs. A. G. Ackerley, F. E. Dische, H. Middleton, and J. H. Rack supplied me with some of the material.

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Experimental Supravalvular Mitral Stenosis in the Dog

A Study of the Pathology of Induced Stenosis, with Special Reference to the Lesser Circulation

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Conflicting reports have appeared in the literature with regard to the pulmonary vascular changes in mitral stenosis. Parker and Weiss,[1] in 1936, described some medial hypertrophy in the small pulmonary arteries. A similar change was noted in 11 of 32 lingular biopsy specimens taken at the time of mitral commissurotomy by Enticknap.[2] Larrabee, Parker, and Edwards[3] noted changes in the pulmonary arterioles in 18 of 20 patients with advanced mitral stenosis. The commonest change described was medial hypertrophy. On the other hand, O'Neal, Thomas, and Hartroft,[4] by careful measurements, were unable to demonstrate medial hypertrophy of the pulmonary arteries in 30 cases of mitral stenosis.

The pathogenesis of the intimal thickening of the pulmonary arteries in secondary pulmonary hypertension is likewise not well established. However, there is increasing evidence that thrombi or emboli with organization may represent the cause of this intimal change.[5-10]

In an attempt to answer some of these questions, it seemed desirable to study the development of pulmonary hypertension in the experimental animal. With this purpose in mind, a controlled degree of supravalvular mitral stenosis was produced in dogs, and physiologic and pathologic studies were undertaken.

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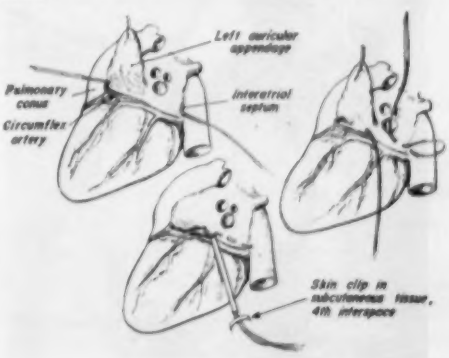
Material and Methods

Thirty-three mongrel dogs were used in this experiment. The animals weighed between 4.8 and 13.8 kg. at the start of the experiment. There was a preponderance of male animals. The dogs were fed a routine diet and were caged separately in an air-conditioned room.

A supravalvular mitral stenosis was produced in all animals by a modification of a technique described by Ellison, Major, Pickering, and Hamilton[11] (Fig. 1). This method did not cause mitral regurgitation, as the mitral valve could still function normally. Likewise, no appreciable myocardial damage resulted except in one dog, in which the left circumflex coronary artery was incorporated in the suture. In eight dogs an azygos vein ligation was performed prior to the production of the mitral stenosis. It was hoped that this procedure would prevent any shunts developing between the pulmonary and bronchial venous circulations. In all animals the supravalvular stenosis was produced over a period of approximately six months by gradually tightening the ligature. All animals survived for more than 100 days after the development of the stenosis. The longest survival time was 760 days, and seven dogs lived for more than a year.

Fig. 1.—Schematic diagram showing method by which a supravalvular mitral stenosis is produced.



Many of the animals had cardiac catheterizations, femoral artery punctures, and oxygen consumption determinations. This allowed us to study cardiac output and pulmonary artery and wedge pressures, and to determine pulmonary and left-sided resistance. Electrocardiograms were obtained on all dogs.

A necropsy was performed on each animal. Blocks of tissue taken from the heart, lungs, spleen, liver, kidneys, adrenals, pancreas, and gastrointestinal tract were fixed in 10% neutral formalin and sections stained with Bullard's hematoxylin and eosin method. Lung sections were also stained with Weigert's elastic tissue stain and counterstained with Van Gieson's trinitrophenol (picric acid) fuchsin mixture. A few sections of liver were stained for connective tissue with Mallory's aniline blue method.

Results

Fifteen animals developed clinical evidence of congestive heart failure, as indicated by ascites and pedal edema (Fig. 2). Many of these dogs required weekly paracenteses. Dyspnea was noticeable in these animals. These dogs gained weight, owing to the accumulation of fluid, whereas the nonedematous dogs showed no appreciable weight changes.

Fig. 2.—Clinical appearance of dog in congestive heart failure. The protuberant abdomen, dilated superficial veins, and nodule in left chest from suture are apparent.

The physiologic changes are to be reported in detail at a later date. Suffice it to say that pulmonary hypertension was produced and there was usually a fall in cardiac output. There was invariably a higher pulmonary artery and wedge pressure in dogs with azygos vein ligation.

At necropsy hydrothorax was noted in six dogs. There were a few adhesions between the visceral and the parietal pericardium near the suture line, but in no instance was there a constrictive pericarditis.

The weight of the hearts was increased in 12 dogs. These heart weights were compared with those of normal dogs and were not considered increased unless they exceeded the expected weight by more than 25%. The increased weight of the hearts was roughly parallel to the duration of the supravalvular mitral stenosis and, to a less evident degree, to the grade of the stenosis.

Right ventricular hypertrophy was observed in 13 animals (Fig. 3). This was believed to exist when the right ventricle was more than one-half the thickness of the left ventricle. Here, also, there was a general correlation between the duration of the supravalvular stenosis and, to a less degree, the grade of stenosis.

The aperture produced by the supravalvular stenosis was less than 5 mm. in diameter in 8 animals (Fig. 4), between 5 and 10 mm. in 17 animals, and between 11 and 15 mm. in 8 animals. Seven dogs had a stenosis with a double lumen. This was produced when the constricting band passed too far to the left. These seven dogs have been included in the appropriate groups shown above, as indicated by the total area of two apertures. The left atrium was usually dilated, and the endocardium was thickened, gray, and opaque.

The left circumflex artery was compressed by the constricting band in one animal, with resultant myocardial infarction. Twenty-three dogs had partial inferior vena caval obstruction by the constricting band. This obstruction apparently contrib-

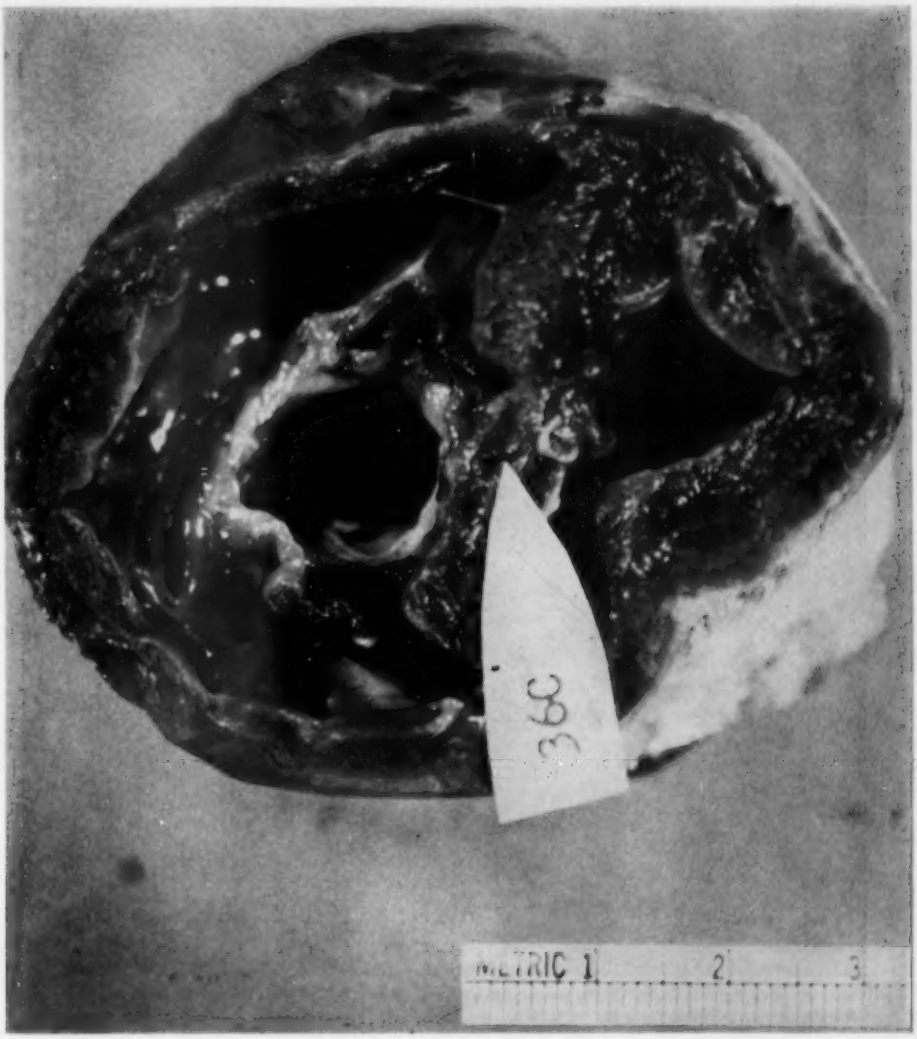


Fig. 3.—Transverse section of the heart midway between the apex and the atrioventricular groove. Hypertrophy of the right ventricle is noted on the left side of the photograph. The arrow points to the interventricular septum.

uted to the ascites and pedal edema; however, in a few dogs there was evidence of congestive heart failure without any inferior vena caval obstruction. Two animals had a bacterial endocarditis at the site of the supravalvular stenosis.

The lungs were usually heavy. There was invariably acute congestion, while pulmonary edema was frequently present. One animal showed three small pulmonary infarcts.

The spleen was appreciably enlarged in seven animals. Infarcts were present in nine spleens. These were generally organizing and not infrequently multiple.

The liver was enlarged in 27 dogs. There was an accentuation of the lobular markings, with a "nutmeg" appearance. There

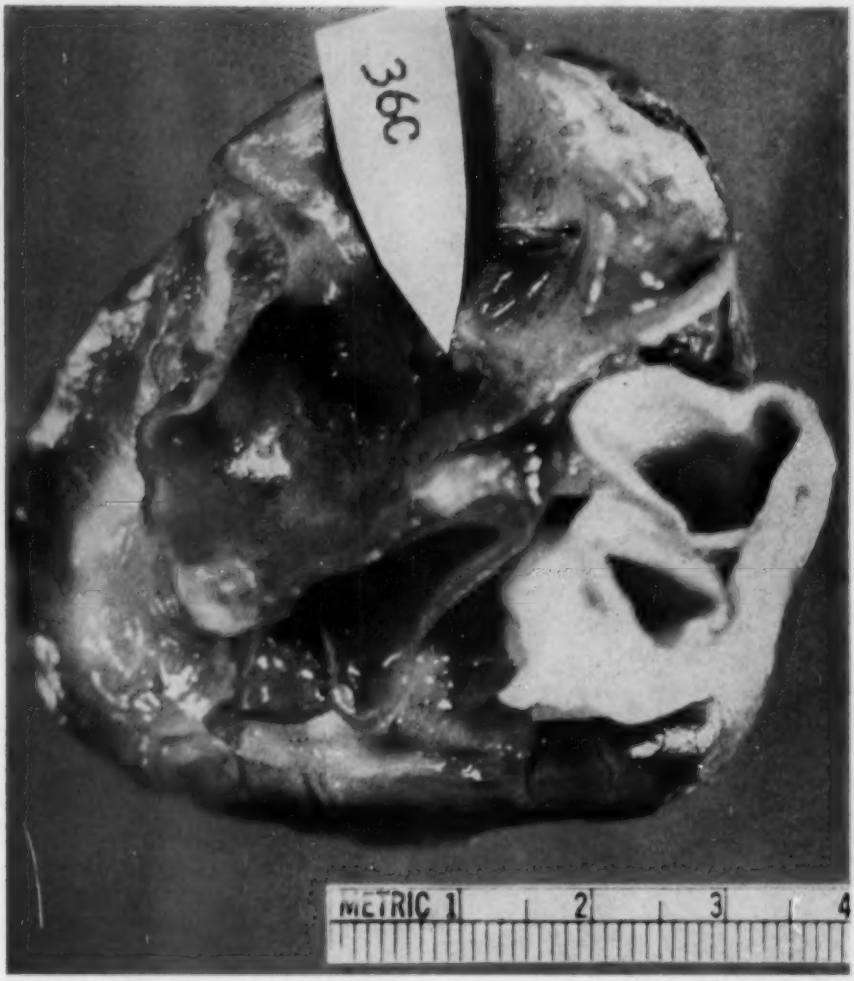


Fig. 4.—Supravalvular mitral stenosis viewed from the left atrial aspect. The arrow is shown at the periphery of the aperture.

was an increase of consistency in nine animals.

The kidneys of seven dogs showed multiple infarcts in various stages of organization. Linear gray streaks in the cortices were frequent, and abscesses were noted in the kidneys of two animals; these were in the two animals with endocarditis.

The other thoracic and abdominal viscera were not remarkable.

Histologic examination of the hearts revealed fibrous thickening of the left atrial endocardium. There were recent and old infarcts of the left ventricle in the dog with the constriction of the left circumflex coronary artery. One dog had a prominent myocarditis in which the larvae of Dirofilaria immitis were identified.

Acute congestion and pulmonary edema were frequently found in the lungs. The alveolar septa showed thickening, but this was due to dilatation of the capillaries, producing a beaded appearance (Fig. 5). No definite fibrous thickening of the alveolar

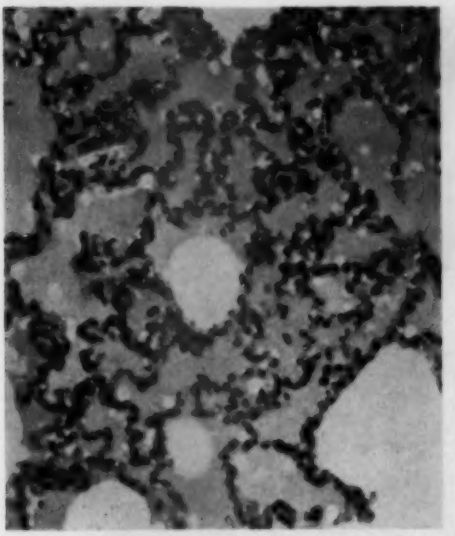


Fig. 5.—Lung. There is congestion of the alveolar capillaries, producing a beaded appearance. Abundant edema fluid is noted in the alveoli. Hematoxylin-eosin stain; reduced to 45% of mag. × 325.

septa was demonstrated. There was a superimposed bronchopneumonia in 14 dogs. Hemosiderin-laden histiocytes were present in the alveoli of two animals (Fig. 6). This feature was more prominent in the anterior portions of the lungs. A rare example of pulmonary alveolar microlithiasis was noted in one dog. Seventeen animals had a moderate heart worm infestation.

Particular attention was paid to the pulmonary blood vessels. Arterioles, varying from 50μ to 150μ, were measured according to the method of Kernohan, Anderson, and Keith.[12] The medial mass was likewise determined in those cases with a decreased lumen-to-wall ratio after the method of O'Neal, Thomas, and Hartroft.[4] Five vessels cut at approximately right angles and associated with respiratory bronchioles were chosen for these measurements. Ten essentially normal dogs were used as controls. The lumen-to-wall ratio in the normal dogs varied from 5.0 to 18.0, with an average of 11.3. The dogs with mitral stenosis had a lumen-to-wall ratio varying from 3.9 to 20.0, with an average of 11.4. In only two dogs was the ratio less than 5.0; one of these animals had a moderately severe heart worm infestation. However, the medial mass was increased in both dogs (Fig. 7). In one animal marked intimal changes were found. These intimal changes are believed to be the result of the organization and canalization of thrombi (Fig. 8). The final result was an eccentric intimal fibrous plaque. The larger arteries in many animals showed the villous intimal thickening associated with D. immitis infestation.

Passive congestion of the liver was present in 32 dogs. This was characterized as severe in 18, moderate in 6, and slight in 8 dogs. Cardiac cirrhosis of slight to moderate degree was present in 12 animals.

Splenic infarcts, showing various stages of organization, were noted in seven dogs. Small abscesses were present in the spleen of one of the animals with endocarditis.

Infarcts were present in the kidneys of seven animals. There was frequently a chronic inflammatory infiltration attributable to worm infestation in many dogs. Abscesses were observed in three dogs.

The adrenals frequently showed focal or diffuse cortical lipid depletion. The pancreas and gastrointestinal tract revealed no changes that could be attributed to the experimental stenosis.

Comment

It was possible to produce a controlled degree of supravalvular stenosis by this method. The part played by the constriction of the inferior vena cava in the pathogenesis of the ascites, liver changes, and pedal edema is difficult to assess; it would seem that the constriction would aid in the development of these changes. However, at the time of cardiac catheterization a catheter could be inserted with ease into the inferior vena cava and no appreciable differences in pressure at the level of the liver and the right atrium were noted.

In all the animals catheterized there was an increase of pulmonary artery and wedge

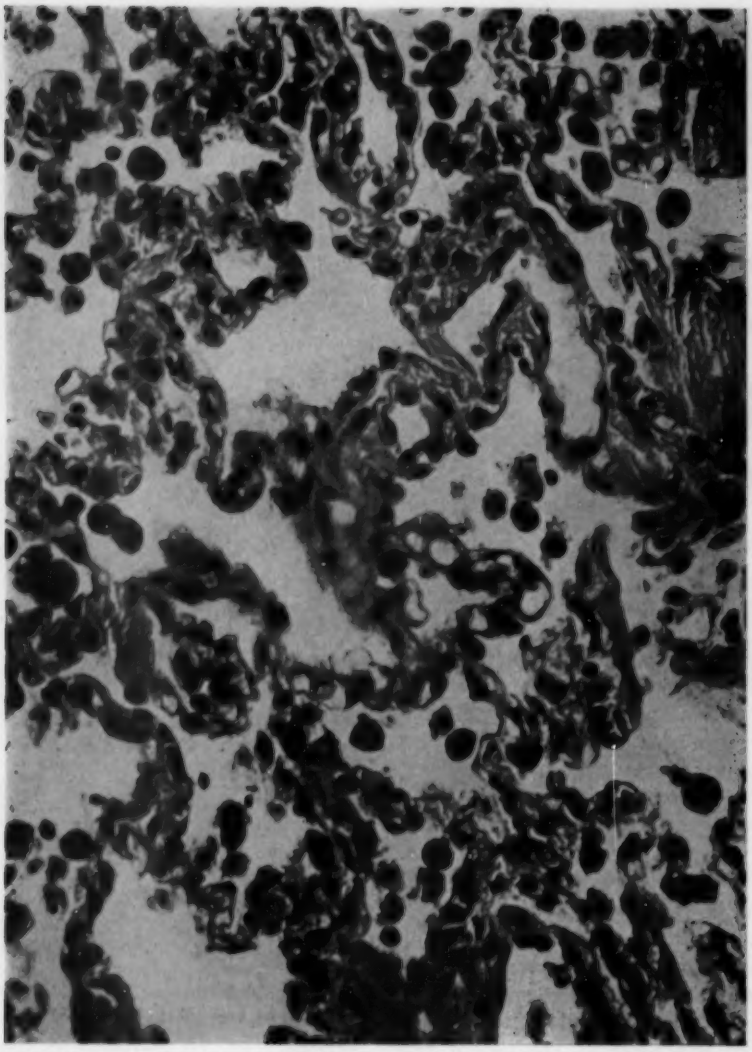


Fig. 6.—Lung. Congestion of alveolar septa and focal aggregates of hemosiderin-laden phagocytes in alveoli. Hematoxylin-eosin stain; reduced to 90% of mag. × 625.

pressures. It would appear that azygos vein ligation prevented any shunting of blood into the bronchial venous circulation and hence accounted for the increased pressures in the lesser circulation.

Anatomic evidence of pulmonary hypertension was apparent in the cardiomegaly and right ventricular hypertrophy of approximately one-third of the dogs. However, changes in the small pulmonary arteries and arterioles were absent. In only one instance was there unequivocal evidence of medial hypertrophy due to pulmonary hypertension alone. This animal survived for 760 days with a supravalvular aperture of 4 mm. The medial hypertrophy in the other dog might have been partially accounted for by the moderately severe heart worm infestation.

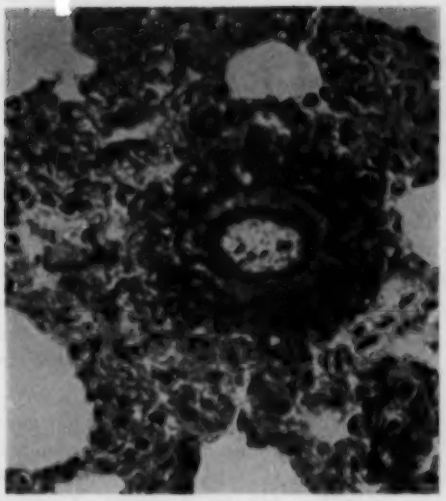
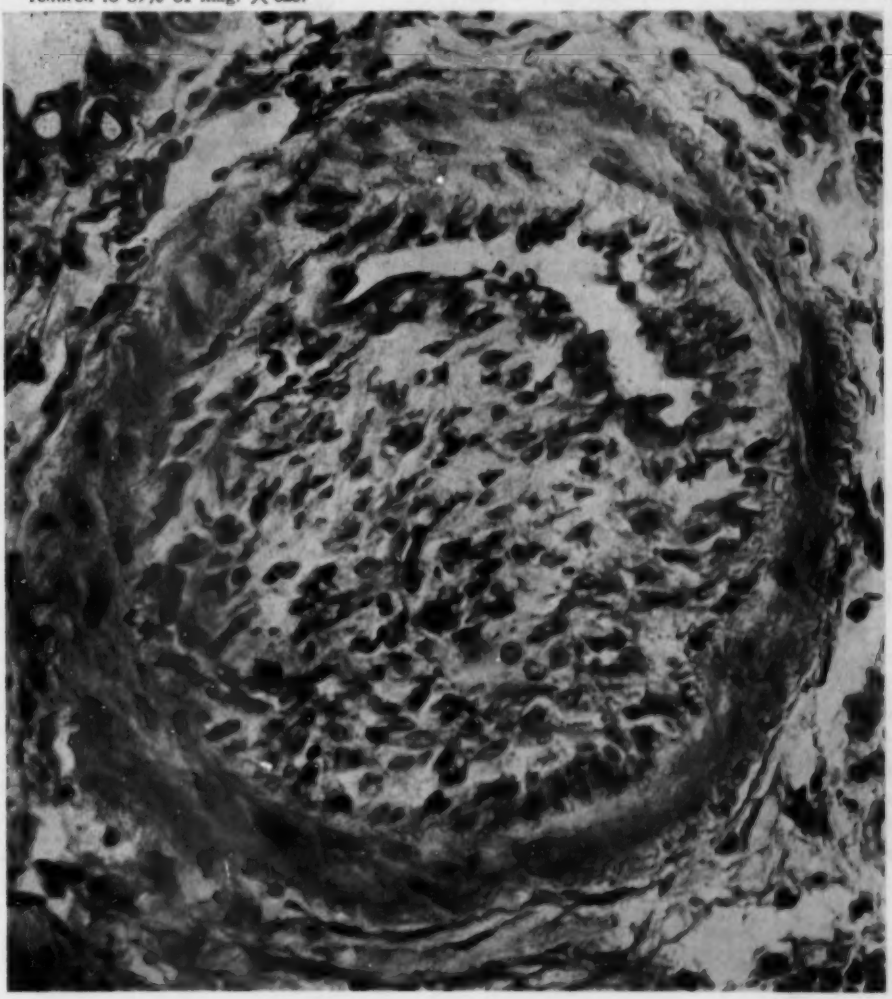


Fig. 7.—Lung. Cross section of small pulmonary artery, showing slight medial hypertrophy. Weigert's elastic tissue stain; reduced to 72% of mag. × 625.

Fig. 8.—Lung. Pulmonary artery showing organizing thrombus. Hematoxylin-eosin stain; reduced to 89% of mag. × 625.



The role played by heart worms in the development of pulmonary hypertension is more difficult to evaluate. That D. immitis infestation may produce congestive heart failure alone is well recognized.[13] Yet the high incidence of heart failure in this group of animals would make heart worm infestation as the major cause improbable. The microscopic changes produced by heart worms are usually easily recognized and can be distinguished from the changes resulting from mitral stenosis.

The finding of marked intimal changes that represented organizing and organized thrombi lends support to the concept of recent workers.[5-10] Work now in progress in this laboratory in which autogenous clots are injected into the pulmonary circulation of dogs with the development of pulmonary hypertension and intimal changes lends further support to this idea.[14]

We believe that the most significant contribution from this study is infrequency of pulmonary vascular changes. It would appear that significant pulmonary hypertension can exist without anatomic changes in the lesser circulation. The increased vascular resistances must be attributed to functional vasoconstriction.

Summary

A method of producing a controlled degree of supravalvular mitral stenosis in dogs is described. By this procedure, it is possible to produce congestive heart failure with pulmonary hypertension. Higher pressures in the lesser circulation could be produced by obliterating the bronchial venous circulation with azygos vein ligation. Cardiomegaly and right ventricular hypertrophy can be produced by this technique. There was generally an absence of anatomic changes in the lesser circulation in the presence of a sustained pulmonary hypertension. The occasional finding of intimal changes attributed to organizing thrombi lends support to the current concepts of pulmonary arteriosclerosis.

Mr. Robert Gary gave technical assistance, and Mr. Nicholas Gagliano Jr. did the photography.

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Thromboembolism and Experimental Systemic Arteriosclerosis

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In recent years several investigators have produced arteriosclerosis of the small pulmonary arteries of rabbits by the repeated intravenous injection of thromboemboli.[1-7] Although the ultimate significance of these experiments is not known, they have already helped to elucidate many problems related to pulmonary arteriosclerosis.

No reports have appeared of the production by thromboembolism of lesions in systemic arteries similar to those associated with thromboembolism in the pulmonary arteries. A technique for producing thromboembolic-induced systemic arteriosclerosis might be useful in investigating problems pertaining to systemic arteriosclerosis in man. The word arteriosclerosis is used herein in its broadest sense to indicate any type of sclerosis of arteries.

Recently, we have developed a method for the production of systemic arteriosclerosis by the repeated injection of a blood clot-suspension into the general circulation. The purpose of this report is to present the results of our initial experiments.

Methods and Material

Eighty young adult New Zealand White rabbits of both sexes were used as experimental animals. They were kept in air-conditioned animal rooms, fed 100 gm. of Purina Rabbit Pellets daily, and offered water as desired.

Whole blood for preparation of a clot suspension was obtained by cardiac puncture from other healthy rabbits. This blood was beaten rapidly with a metal rod. The resultant clot consisted chiefly of fibrin, but there were some enmeshed erythrocytes. The clot was separated from the fluid portion of the blood and placed in a Waring Blendor. Enough isotonic saline was added to the clot to produce a mixture with the same volume as that of the whole blood from which the clot had been obtained. The Waring Blendor was operated at high speed for 5 to 10 minutes, breaking the clot into fragments that would pass readily through an 18-gauge needle but not readily through a 20-gauge needle. For most groups of rabbits (as indicated below) 2 or 3 drops of India ink were added for each 100 ml. of clot suspension to facilitate location and identification of thromboemboli.

The clot suspension was injected through an 18- or 20-gauge needle into the heart or arteries of recipient rabbits. For the cardiac injections an 18-gauge needle was introduced through the intact thorax into the left ventricle. The left ventricle could usually be readily identified by the pulsation and the color of the blood. However, occasional injections were undoubtedly given into the right ventricle or other chambers. It quickly became apparent that quantities of clot suspension larger than 1 ml. were often fatal; so most injections were limited to 1 ml. The number of intracardiac injections given to animals used in the experiments reported herein varied from one to seven, and the length of time between the first injection and death varied from 2 to 57 days. Animals that died immediately after the first injection were not included in the experiment. Intra-arterial injections were forced through a 20-gauge needle into the central arteries of the rabbit's ears with the clot directed distally. Only one arterial injection, consisting of clot from 3 ml. of blood, was given in each ear.

In addition to 25 rabbits on regular diets that received intracardiac or intra-arterial clot suspension marked with ink, several groups were established to investigate special aspects. These included (1) 5 rabbits receiving intracardiac clot suspension not marked with ink, (2) 13 rabbits receiving supplementary cholesterol feedings (2 gm. daily)

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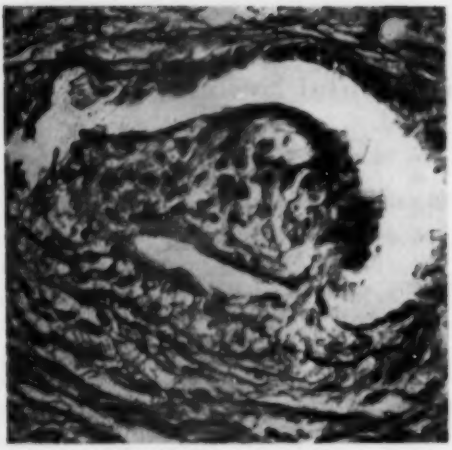


Fig. 1.—Organizing thromboembolus in a coronary artery of a rabbit that received four intracardiac injections of clot suspension. This animal received oral cholesterol daily for 49 days. Aldehyde fuchsin-Van Gieson stain; reduced to 94% of mag. × 400.

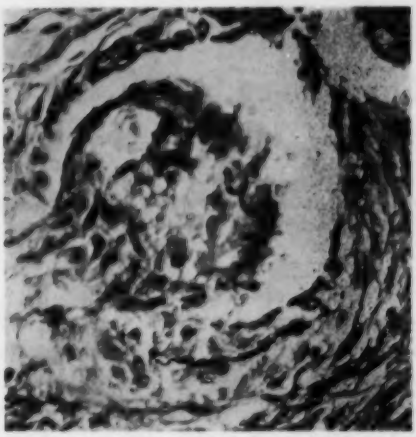


Fig. 2.—Another organized thromboembous in a coronary artery of the same rabbit shown in Figure 1. Aldehyde fuchsin-Van Gieson stain; reduced to 88% of mag. × 460.

with and without intracardiac injections of marked (ink) or unmarked clot, (3) 3 rabbits receiving intracardiac ink but no clots, (4) 5 rabbits bled repeatedly from the heart without any injections, (5) 3 rabbits into whose ear arteries saline was injected at pressures from 200 to 500 mm. of mercury, (6) 25 rabbits whose ear arteries had not been injected with anything, and (7) 6 rabbits whose ear arteries were injected with clot suspension after transparent ear chambers had been inserted (using the technique of the Clarks).[5]

All animals either died during the course of the experiment or were killed with intravenous formalin. Autopsies were performed and the contents of the thoracic and peritoneal cavities were examined, special attention being directed to the heart and kidneys. Selected portions of tissue were fixed in 10% formalin. After fixation multiple blocks of tissue were taken for paraffin sections from the heart, lungs, kidney, liver, and stomach. The paraffin sections were stained with hematoxylin and eosin and with an aldehyde fuchsin-Van Gieson-iron hematoxylin stain that has been used by us to demonstrate elastic and connective tissue.[6] A few selected blocks were taken for frozen sections, and these were stained with oil red O.

Fig. 3.—Eccentric lesion of a coronary artery with a tail suggesting its thromboembolic origin. This rabbit received four injections of clot suspension and cholesterol for 37 days. Aldehyde fuchsin-Van Gieson stain; reduced to 94% of mag. × 210.

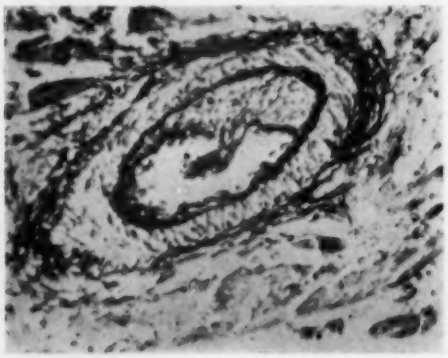
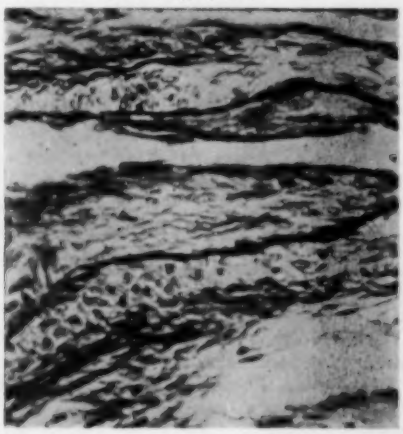


Fig. 4.—Fibrous intimal lesion in a coronary artery of a rabbit that received five intracardiac injections of clot suspension and cholesterol for 49 days. Aldehyde fuchsin-Van Gieson stain; reduced to 94% of mag. × 230.



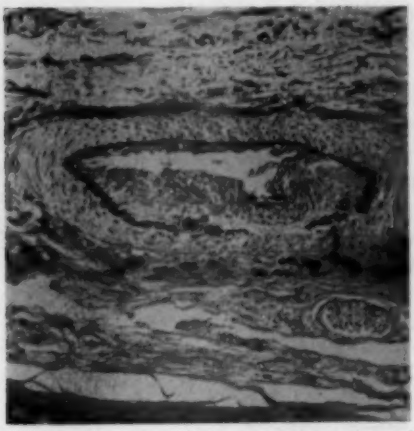


Fig. 5.—A fibrous intimal arterial lesion with fracture of the internal elastic lamina from the ear of a rabbit that received three injections of clot suspension and cholesterol for 34 days. India ink particles are present in the intimal lesion and in the adventitia. Aldehyde fuchsin-Van Gieson stain; reduced to 94% of mag. × 380.

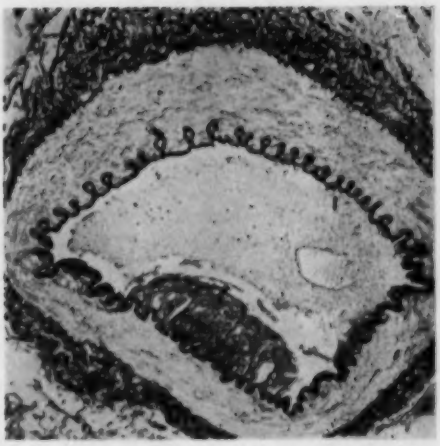


Fig. 6.—A mound-like fibrous intimal lesion from a renal artery of a rabbit that received four injections of clot suspension and cholesterol for 49 days. Aldehyde fuchsin-Van Gieson stain; reduced to 88% of mag. × 225.

Results

The 25 rabbits on normal pellet diets that jections of 1 ml. of clot suspension marked were given one to seven intracardiac in- with ink had no gross lesions in their aortas or other large arteries. Myocardial infarcts were observed in 16 of the 25 rabbits, renal infarcts in 15, and a gastric infarct in 1. Infarcts were not observed in other organs, but our attention was directed particularly to the heart and kidneys. The brains were not examined.

Microscopic examination confirmed the presence of myocardial, renal, and gastric infarcts, of varying ages. Some were quite

Fig. 7.—An eccentric fibrous intimal lesion from a gastric artery of a rabbit that received seven injections of clot suspension and no cholesterol. Aldehyde fuchsin-Van Gieson stain; reduced to 94% of mag. × 280.

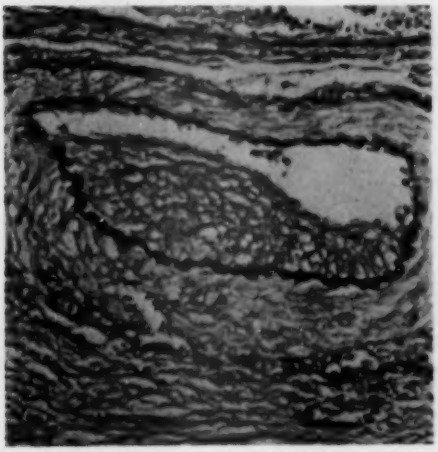
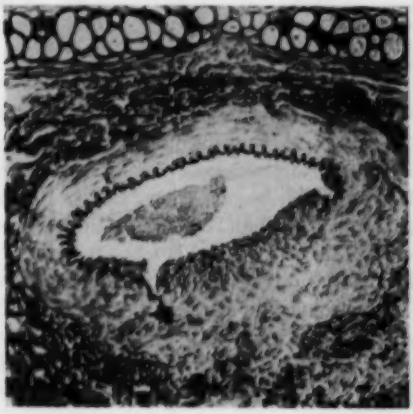


Fig. 8.—A fibrous intimal lesion from an ear artery of a rabbit that received a single injection of clot suspension into the central artery of the ear and no cholesterol. India ink particles are present in the adventitia. Note the discontinuity of the internal elastic lamina in the lower half of the picture. Aldehyde fuchsin-Van Gieson stain; reduced to 94% of mag. × 210.



recent, and in others the normal tissue had been entirely replaced by fibrous tissue. Small collections of fat were present in many of the myocardial infarcts. Rather extensive calcium deposits were observed in some, but not all, of the myocardial and renal infarcts.

Histologically, arterial lesions of two general types were observed. One type was a clearly recognizable organized or organizing thrombus, partially or completely occluding the lumen of the containing vessel. Many of these had multiple channels of recanalization. The other type of lesion was characterized by fibrous thickening of the intima, forming an eccentric or more or less concentric plaque. The plaques consisted of fibroblasts and collagen. Vascularization of, or hemorrhage into, the plaques was not observed in our sections. None contained hemosiderin, ceroid, or calcium. A few of the lesions were stained for fat, but none was found. The dividing line between plaques and clearly recognizable thrombi was not a sharp one, but most lesions could be classified as one or the other. Although the internal elastic membrane was usually intact, it was occasionally broken beneath both types of lesions. Many

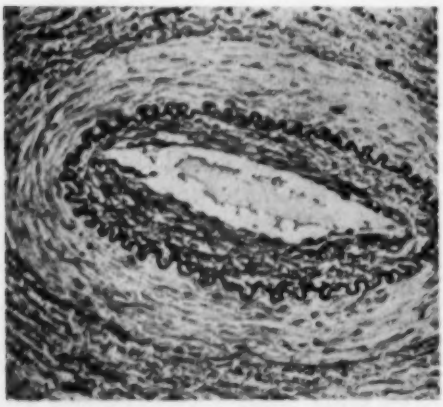


Fig. 9.—A concentric fibrous intimal lesion from an ear artery of a rabbit that received four intracardiac and one intra-arterial injection of clot suspension and no oral cholesterol. Aldehyde fuchsin-Van Gieson stain; reduced to 88% of mag. × 345.

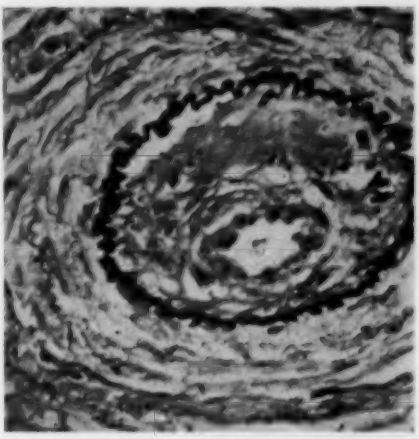
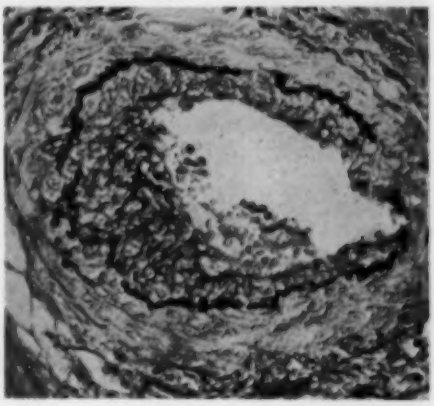


Fig. 10.—An arterial lesion from the heart of a rabbit that received three intracardiac injections of clot suspension and cholesterol for 34 days. Ink particles are present in the intimal lesion. Aldehyde fuchsin-Van Gieson stain; reduced to 94% of mag. × 270.

lesions contained only a few particles of ink, although large quantities were present in some and none in others. The changes in the ear arteries of the rabbits that received intra-arterial injections of clot were similar to those described above in visceral arteries.

The rabbits that were given intracardiac injections of clot suspension not containing ink developed arterial lesions and infarcts

Fig. 11.—A fibrous intimal lesion in a coronary artery of a rabbit that received seven intracardiac injections of clot suspension and no cholesterol. Aldehyde fuchsin-Van Gieson stain; reduced to 88% of mag. × 390.



in their hearts and kidneys indistinguishable from those described above except for the absence of ink particles.

No arterial lesions or infarcts were observed in the rabbits that were given intracardiac or intra-arterial injections of ink solution or that were simply bled from the heart. No lesions were observed in arteries of the ears of the 25 rabbits that had not been injected intra-arterially. Furthermore, no lesions of any kind were observed in the arteries of the ears of the rabbits that had received intra-arterial injections of saline at high pressure (200-500 mm. of mercury as measured directly with a needle in the distal portion of the artery).

Rabbits that received cholesterol in their diets without intra-arterial or intracardiac injections developed the typical arterial lesions that have been described by others. These lesions consist largely of subendothelial collections of fat-containing mononuclear cells and extracellular fat.

Rabbits that received intracardiac or intra-arterial injections of clot suspension and cholesterol-supplemented diets developed the classical type of lesions associated with cholesterol feeding (in the absence of clot) and the types of lesions that have been described above in rabbits that received intracardiac injections of clot but no cholesterol. The few thromboembolic-induced lesions that were stained with oil red O showed a much higher content of fat than similar lesions in rabbits that had not received cholesterol in their diets. Also, the myocardial infarcts contained more fat in the cholesterol-fed rabbits than in those that did not receive cholesterol. The grossly recognizable aortic lesions and some of the lesions in the small arteries were similar to the cholesterol arteriosclerosis in the rabbits that were not injected with clot suspension.

Transparent ear chambers were inserted into the ears of several rabbits over the central artery, making it possible to view with the microscope an area of tissue approximately 1 cm. in diameter. Clot marked with ink was injected into the central artery of the ear proximal to the ear chamber. Enough clot was injected to produce numerous visible thromboemboli in the ear chamber. These thromboemboli tended to be trapped at the branches of arteries, but eddy currents usually swept them farther down rather rapidly until they reached a part where the vessel was completely plugged. Even then the thromboemboli usually did not remain stationary but were swept back and forth by the eddy currents. During movement pieces of clot were constantly being separated from the main mass and swept farther down the vessel, where the process was repeated until the fragments became small enough to pass through the capillaries or else passed out of the ear chamber. Most of the thromboemboli disappeared from view within an hour. By the end of 24 hours we were seldom able to see more than one or two fragments of the clot, and these usually disappeared in a few more days. However, we were able to observe one thromboembolus in a branch of the central artery for a period of 10 days. At the beginning of the period the artery was completely occluded. It gradually became recanalized, and by the end of the period of observation the channel appeared to be of normal size. The rabbit was killed, and sections were taken of the artery that had contained the thrombus. Ink was found in the adventitia around the vessel, but otherwise this particular vessel appeared normal, with no intimal thickening. However, other vessels in this and other ear chambers had fibrous intimal thickening of arteries similar to that described above in rabbits without ear chambers that were given intra-arterial injections of clot.

Comment

A method is described for producing sclerotic lesions in the systemic arteries of rabbits by the repeated injections of thromboemboli. This method may prove to be useful in the investigation of many special problems pertaining to arteriosclerosis in man.

Several types of microscopic arterial lesions appearing to arise from thromboemboli were produced in this experiment. Some of these lesions were clearly recognizable organized thromboemboli. Others were indistinguishable from some sclerotic lesions found in the small systemic arteries of man. Still others appeared to represent a transition stage between organized thrombi and plaques. However, no gross atherosclerotic changes appearing to result from thromboemboli were produced in the aorta or elsewhere.

The systemic arterial lesions produced in this experiment are similar to those that have been produced in the pulmonary arteries by thromboembolism.[1-7] Vascularization of plaques has not been observed in small systemic or pulmonary arteries. Apparently the cells in small plaques receive their nourishment directly from the blood in the lumen of the containing vessel. The absence of hemosiderin in these plaques may be accounted for on the basis of the lack of vascularization of the plaques and the fact that the thromboemboli injected consisted almost entirely of fibrin.

The addition of small amounts of India ink to the clots made it possible to identify with certainty the resulting arterial lesions. Although India ink consists of carbon particles which are relatively inert, it could have had some additive effect on the lesions. However, many lesions contained no ink particles, even in the rabbits that received clot marked with ink. Also, arterial lesions were produced in rabbits that received clot not marked with ink. The lesions that did not contain ink were quite similar to those that contained ink.

We were suprised to find that thromboemboli injected into the left ventricle passed readily into the coronary arteries, as indicated by the high incidence of myocardial infarcts. Perhaps myocardial infarcts in human patients result more frequently on the basis of thromboemboli than is commonly believed.

The observations made of thromboemboli through the transparent ear chamber suggest that most thromboemboli disintegrate rapidly and are not incorporated into the vessel walls. We do not know what factors determine why one thromboembolus will become attached to a vessel wall and result in a fibrous intimal lesion and another will disintegrate. The factors could be in the thromboembolus, in the vessel wall, or in the blood stream.

We have demonstrated in previous experiments with thromboembolic-induced pulmonary arteriosclerosis that the quantity of lesions can be influenced by certain types of fatty meals.[7] Meals of butter and oleomargarine (saturated fats) resulted in a marked increase in the number of lesions in the pulmonary arteries associated with thromboemboli, whereas meals of corn oil (an unsaturated fat) had no effect. We do not yet know the effect of such fatty meals on thromboembolic-induced systemic arteriosclerosis. However, the appearance of the basic lesions is similar in the pulmonary and systemic arteries of rabbits.

We believe that the most important aspect of the experiment reported herein is that a new method has been introduced for the investigation of the relationship of lipidosis, thrombosis, and fibrosis in systemic arteries. Further experiments are now in progress designed particularly to test the effect of cholesterol feeding on thromboembolic-induced systemic arterial lesions.

Summary

In recent years many investigators have produced arteriosclerosis in small pulmonary arteries by the repeated intravenous injection of blood clots. In the experiment reported herein similar lesions have been produced in systemic arteries by intracardiac (left ventricle) and intra-arterial injections of blood clot.

The addition of cholesterol to the diets of rabbits receiving intracardiac clots resulted in adding fat to the thromboembolic-induced lesions in the small systemic arteries. However, thromboemboli were not found in or on the cholesterol lesions in the aorta.

Experiments are in progress designed to test further the effect of combining cholesterol and thromboembolic arterial lesions.

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Gliomatosis Cerebri

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Diffuse neoplastic transformation of glial elements within the brain and brain stem is rare in patients dying of brain tumor. The term "gliomatosis cerebri" given to this bizarre type of neoplasia appears most appropriate.[1] Similar examples of this process have been described by such terms as "diffuse glioma of the brain," "gliomatous hypertrophy," "diffuse systematic overgrowth of glial apparatus of the brain," "blastomatous type of diffuse sclerosis," and "central diffuse schwannosis." Other names include "cerebral glioblastosis,"[2] "patchy blastomatous infiltration of the central nervous system,"[3] and "astrocytoma diffusum."[4]

The process has been thought to represent a blastomatous type of malformation [1,3,5-9] and to be related, therefore, to some dysgenetic abnormality of the neuroglial system. Others [4,10,11] have considered it to be a true neoplasia of the astrocytic type. Scheinker and Evans [2] suggested that the pathologic process was intermediate between that of a true glioma and a blastomatous type of Schilder's disease. Scheinker [12] later considered the process as a form of demyelinating disease of the brain.

The controversy centers around the long-disputed question of a possible relationship among Recklinghausen's disease, the central neurinoma of Josephy, glioma of the optic nerve and pons, and certain other types of glioma that possibly bear morphologic resemblance to the cases we are about to report. Nevin [1] likened gliomatosis cerebri to a blastomatous malformation of the Recklinghausen-disease type. He suggested that gliomas of the optic nerve and pons were of the same nature and considered that the explanation for the origin of the heterogeneous cells seen in these processes lay in a dysgenetic abnormality of the neuroglial system, resulting in a widespread blastomatous malformation. Nevin concluded that the process was composed of undifferentiated neuroglial cells.

Others [3,8,9] thought that a central blastomatous growth of Schwannian lemmoblasts accounted for the diffuse overgrowth under consideration. With the experimental demonstration by Harrison [13] of the Schwann-cell origin from the ganglionic portion of the neural crest, a basis for the lemmoblastic development of Josephy's [14] solitary central neurinoma was afforded. Later, with the embryologic assumption that Schwann's cells, or lemmoblasts, might arise from neurogliocytes or indifferent cells of Schaper located anywhere in the central nervous system, a basis for Schwannian overgrowth occurring within the substance of the brain was suggested.[3] This also offered an opportunity to integrate the previously mentioned varied pathologic processes into one common histogenetic abnormality.

On the contrary, Bailey and Cushing [15] and Cox [16] regarded the central neurinoma as a unipolar spongioblastoma; Russell and Bland [17] showed later by means of tissue culture that both these tumors in reality were composed of piloid astrocytes. Therefore, an astrocytic basis for the process was suggested. Russell and Cairns [18] pointed out that the morphologic aspects of astrocytes may be influenced by their environment; thus, they may assume an elongated form

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in the midst of white-fiber tracts and a stellate form when located in gray matter. We subscribe to this concept.

In spite of the many opinions held regarding gliomatosis cerebri, current trends suggest that it represents a neoplastic glial process [11,19] that is probably astrocytic in type.[10]

We wish to present two examples of diffuse cerebral gliomatosis that appear to bear resemblance to the processes already described.

Report of Cases

CASE 1.—An 18-year-old woman was admitted to the hospital in January, 1946, with a history of increasing frontal headache, beginning the previous autumn. She awakened one morning a month prior to admission with severe headache and vomiting. She was hospitalized elsewhere, and some relief was obtained by means of repeated spinal puncture. Diplopia was noted 10 days later, and this persisted until her admission here. Two days prior to admission, tinnitus was noted in the right ear.

The patient appeared chronically ill. Results of general physical examination were within normal limits. Neurologic examination showed a stiff neck (+2 on the basis of +1 to +4) and a positive Lasègue sign. Generalized hyporeflexia was present. The plantar responses were normal. Questionable facial weakness was present on the left, and almost complete palsy of the right abducens nerve was noted.

Routine laboratory studies of the urine and blood showed nothing abnormal. Roentgenologic studies of the skull disclosed slight decalcification of the posterior clinoid processes, probably secondary to increased intracranial pressure.

The visual acuity was 6/6 bilaterally, and 2 D. of papilledema was measured bilaterally. Visual-field examination disclosed no abnormalities. Electroencephalography disclosed a Grade 2 delta rhythm arising from the right occipital region.

Because of increasing severity of the headache and general deterioration in the condition of the patient, ventriculography was performed. A total of 30 ml. of clear ventricular fluid was removed; it contained 45 mg. of protein per 100 ml. Moderate symmetric dilatation of the lateral and third ventricles was noted. The aqueduct and fourth ventricle were not visualized. Because of the possibility of an obstructing mass in the posterior fossa, the patient underwent suboccipital craniectomy the same day. Increased intracranial pressure was evident, with minimal herniation of the cerebellar tonsils. The fourth ventricle was dilated, and a silver probe passed through the aqueduct revealed no obstruction. The cerebellar hemispheres were normal, as were the cerebellopontine angles.

Her headache was greatly relieved after this procedure. By the fifth day after operation, the choked disks measured 1 D. bilaterally and the right abducens palsy was almost totally gone. She was dismissed as symptomatically improved 18 days after operation.

Correspondence during April, 1946, revealed that her vision was progressively failing, that she suffered violent headaches, and that the site of decompression was bulging severely. She died in July, 1946. Necropsy limited to the skull was done elsewhere. The brain was forwarded to the clinic for study.

Gross Examination

The brain showed diffuse enlargement of the right hemisphere (Fig. 1*a*). This enlargement was most apparent within the white matter of the hemisphere and was associated with pronounced flattening of the convolutions over the surface. Relative thickening of the corpus callosum was present. The corticomedullary junctions were less distinct in the regions of maximal swelling than elsewhere. Gross infarction or necrosis was absent. The leptomeninx appeared unduly adherent in isolated zones.

Microscopic Examination

Neoplastic glial elements were found throughout the cerebral hemispheres and brain stem. Although collections of cells were greater in some regions than in others, they were found in all the sections examined. Similarly, involvement was greater throughout the white substance than in the cortex (Fig. 1*b*). In some regions cellular activity within the cortex and that in the underlying white matter were equal in degree. As might be expected, the extent of demyelination (Weigert's stain) and damage to neurofibrils (Bodian's stain) was directly related to the intensity and degree of neoplastic cellularity. Neurons were well preserved, and there was no suggestion of embryonic neuronal forms. Astrocytic glial proliferation was striking in the more cellular zones, especially in certain marginal regions of the cortex (Mallory's phosphotungstic acid hematoxylin stain). The astrocytic nature of the neoplastic cells was

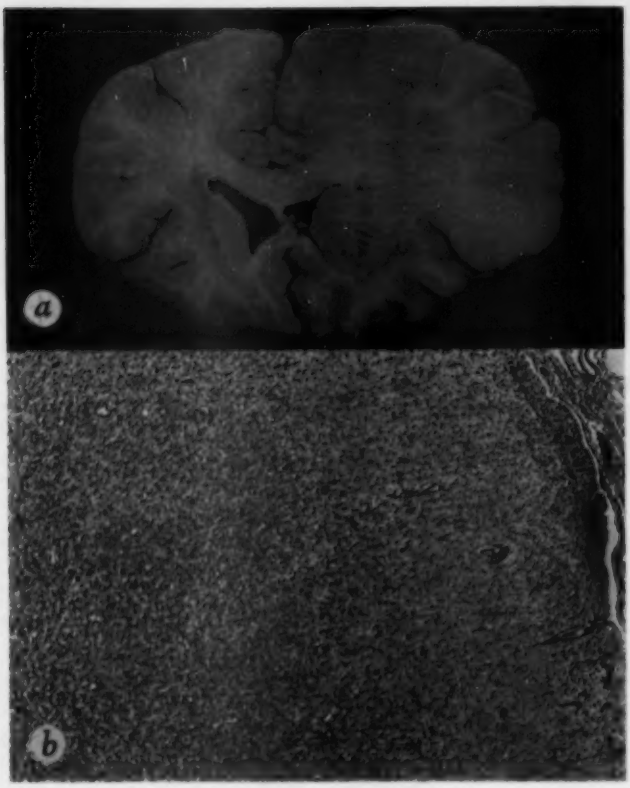

Fig. 1 (Case 1).—(*a*) Coronal section showing pronounced enlargement of the right frontal region. Widening of the gyri and enlargement of the right caudate nucleus are present. The corpus callosum is thickened. (*b*) Right frontal lobe showing relatively greater cellularity in white matter beneath the cortex. Marginal gliosis and leptomeningeal invasion by neoplastic cells are evident. Perivascular cuffing can be seen within the cortex. Hematoxylin and eosin; ×60.

verified by Cajal's gold sublimate and Hortega's triple-impregnation staining techniques.

Cellularity was most pronounced in the corpus callosum, the basal ganglia, including the thalami, the internal capsule, the inferior frontal regions on both sides, and the parieto-occipital lobes. Subpial collections of tumor cells were prominent in these regions of maximal activity adjacent to the cortex. These subpial collections were associated with intense gliosis of the marginal zone of the cortex; although present in some degree in practically all the sections studied, they rarely invaded the leptomeninx (Fig. 1*b*). Cortical involvement was greatest in the regions of maximal subpial and leptomeningeal involvement, and it was in these parts of the cortex that perivascular cuffing and satellitosis of neurons by neoplastic cells were most striking.

Pronounced pleomorphism of the tumor cells was observed. Occasional protoplasmic astrocytes were seen, although these cells usually were confined to small zones of necrosis (Fig. 2*a*). These zones usually were related to thrombosed vessels. The typical cell of the process generally was an elongated one that, within the long white-fiber tracts, characteristically assumed the form of a polar spongioblast (Fig. 2*b*). Although elongated cells also were observed within the cortex, they tended to assume the more conventional astrocytic forms within the gray substance. Of particular interest was the presence of monster cells and multinucleated forms reminiscent of cells encountered in the more malignant forms of astrocytoma (glioblastoma multiforme). Mitotic figures and chromatin variations characteristic of malignant glial

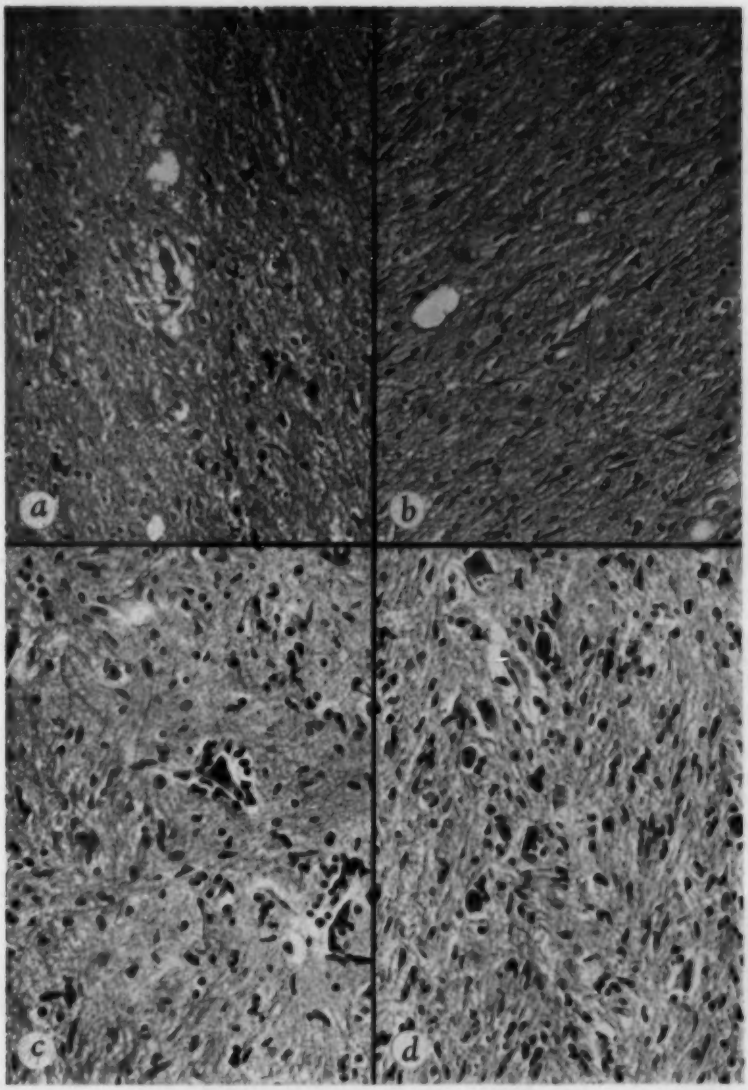

Fig. 2 (Case 1).—(*a*) Left parietal lobe. Neoplastic cellularity is less intense in this region. Protoplasmic astrocytes are interspersed with polar spongioblasts, mature astrocytes, oligodendrocytes, and scattered giant forms. (*b*) Left frontal lobe. Typical polar spongioblasts are seen within the white-fiber tracts; astrocytes, some of protoplasmic type, also are present. (*a*) and (*b*) Hematoxylin and eosin; × 155. (*c*) Left basal ganglion showing perineuronal and perivascular cuffing, with malignant features of pleomorphism, hyperchromasia, and mitotic figures. Hematoxylin and eosin; × 200. (*d*) Mesencephalon. Extensive malignant glial involvement of the brain stem was found. Hematoxylin and eosin; × 210.

tumors were noted throughout (Fig. 2*c* and *d*).

Neoplastic involvement within the mesencephalic, pontine, and medullary levels was confined to aggregates of tumor cells in relation to the long-fiber tracts and could not be found below the upper medullary level. No subpial collections were observed

in this region, and cerebellar involvement was confined to the central nuclear masses.

CASE 2.—A 20-year-old housewife was admitted to the hospital in April, 1952, with a six-week history of forgetfulness and loss of memory. The onset of these symptoms occurred three weeks after delivery of a normal child. Two weeks prior to admission, she had been studied elsewhere and had received five electroshock treatments, without evident improvement. At about the same time progressive frontal headache had been noted. Two days before admission, paresthesia involving the right arm and leg had appeared, associated with some weakness in the right leg.

Results of general physical examination were not remarkable. Neurologic examination showed paresis of the right arm and leg (—2 on the basis of —1 to —4) and slight weakness of the right facial musculature. Hyperreflexia (+2) of the deep tendon reflexes on the right was noted, as was a positive Babinski response on this side. Mild ataxia was present when she walked. Suppression of the ability to perform rapid associated movements on the right was observed. Sensory testing was difficult because of her poor attention; however, a suggestive diminution in response to pinprick was present over the entire right side of the body.

Results of routine laboratory studies of blood and urine were essentially normal. Roentgenologic examination of the skull showed no abnormalities.

Visual acuity was 14/21 on each side, and there was no evidence of choked disks. Visual-field examination showed complete right homonymous hemianopsia. Electroencephalography disclosed a Grade 3 delta focus in the left frontotemporal region, indicative of an acutely destructive lesion involving this region.

Pneumoencephalography gave evidence of incomplete filling of the left lateral ventricle. Questionable shift of the ventricular system to the left was noted, and a normal amount of air was present over the surface of the hemispheres. Results of examination of the cerebrospinal fluid were normal, the content of protein being 25 mg. per 100 ml.

In the absence of definite localization of a space-occupying lesion on the pneumogram, it was decided to observe the course of the patient. She returned six weeks later. In the interim, her condition had progressively deteriorated, with increasing right hemiparesis, increasing memory difficulty, and severe headache. Two weeks prior to her return aphasia had developed. Neurologic examination on this second admission showed complete hemiparesis, with a positive Babinski response on the right. She was unable to walk, and other testing was limited because of sensorial clouding and complete aphasia.

The electroencephalogram showed progression of the left frontotemporal delta focus. Left frontotemporal craniotomy was performed. The surgeon reported no evidence of increased intracranial pressure when the dura was opened. Softening of the superior temporal gyrus was noted, and exploration was performed at this site. A nondemarcated tumor was encountered at a depth of 2.5 cm. Tissue taken from this region showed a diffuse Grade 4 astrocytoma. A small amount of the brain was removed to afford partial internal decompression. The patient did not

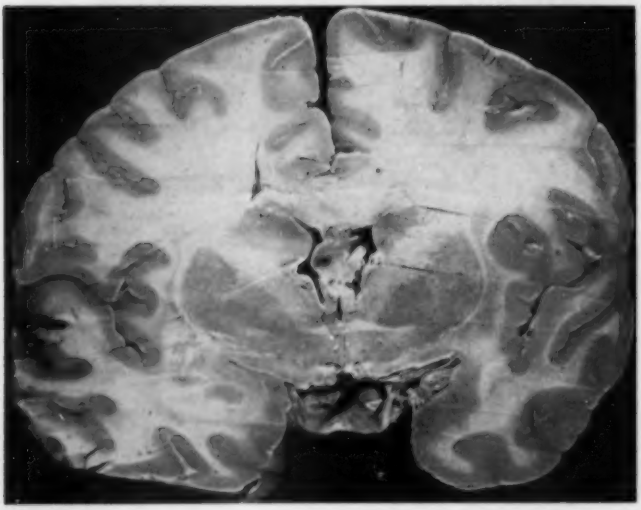

Fig. 3 (Case 2).—Coronal section showing enlargement in the left frontotemporal regions, much of which undoubtedly is due to surgical exploration. Note thickening of the corpus callosum, fornices, and left caudate nucleus. The corticomedullary junctures are distinct.

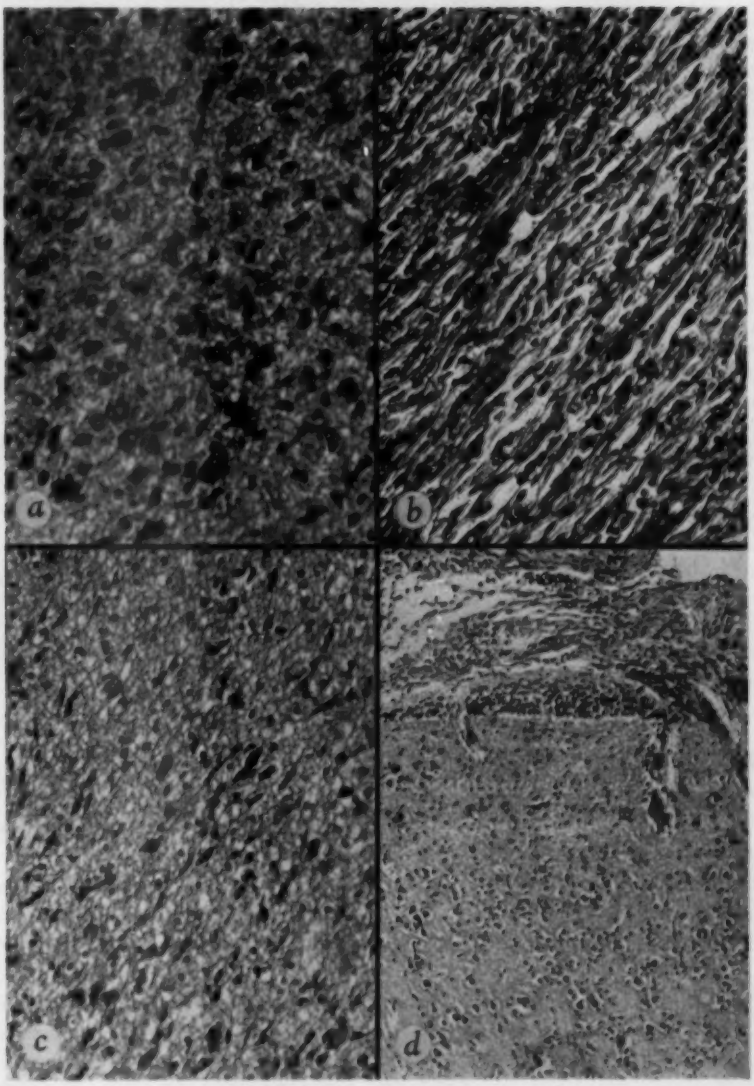

Fig. 4 (Case 2).—(*a*) Right basal ganglion. Note pronounced cellularity with striking pleomorphism, hyperchromasia, and mitotic figures; this is the pattern of a highly malignant astroglial neoplasm. Hematoxylin and eosin; × 150. (*b*) Corpus callosum. Streams of polar spongioblastic forms show orientation between the white-fiber tracts. Note mitotic figures. Hortega's silver carbonate stain; × 200. (*c*) Left frontal lobe. Note pleomorphic cellular pattern with polar spongioblastic forms, multinucleated giant cells, protoplasmic astrocytes, and prominent oligodendroglia. Mallory's phosphotungstic acid hematoxylin stain; × 330. (*d*) Right parietal lobe. The cortex with attached leptomeninx shows invasion of the latter by tumor cells, striking subpial marginal gliosis, perivascular cuffing, and perineuronal satellitosis by neoplastic cells. This degree of leptomeningeal invasion was found confined to isolated regions and was not uniform in extent. Hematoxylin and eosin; × 100.

respond and died the next day of the effects of medullary compression.

Gross Examination

Pronounced flattening of the convolutions and narrowing of the sulci were noted. The operative site extended into the left frontotemporal region. Multiple coronal sections of the brain showed moderate cerebral edema, especially at the site of surgical exploration (Fig. 3). Diffuse thickening of the corpus callosum was present, and small zones of translucency were scattered throughout the white matter of the cerebrum, suggesting tumor. The fornix was thickened bilaterally, and moderate enlargement of the head of the caudate nucleus on the right side was present. There was no region of the brain in which maximal neoplastic involvement was suggested.

Microscopic Examination

Widespread infiltration of the brain by neoplastic glial elements was seen. As in the preceding case, the presence of mitotic figures, polychromasia, and pleomorphism suggested the presence of a malignant glioma (Fig. 4*a*). Both the cortex and the underlying white matter showed varying degrees of involvement; however, a tendency was noted in this case for more striking involvement of the cortex. Dense neoplastic involvement of the corpus callosum was found, with elongated polar cells, so-called polar spongioblasts (Fig. 4*b*). This same type of cell was encountered in other portions of the white matter, although a tendency toward the more frequent occurrence of recognizable astrocytic forms was found in the less compact zones of the white substance. Mitotic figures were frequent, as were multinucleated tumor cells and monster cells. Of interest was a tendency toward more striking evidence of edema and protoplasmic astrocytic forms within the white substance in this case (Fig. 4*c*).

The previously mentioned marginal glial changes were striking in this case (Fig. 4*d*). Gliosis extended through the pial limiting membrane into the leptomeninx. As in the first case, this marginal change usually occurred over zones of greater cellularity. Dense perivascular cuffs and perineuronal satellitosis were observed (Fig. 4*d*). Of interest was the presence in some regions of the cortex of many giant cells; some of these formed perfect circles, and others showed grotesque arrangement in clumps of monster cells (Fig. 5*a*). All gradations of astrocytic activity were observed within the cortical regions.

The corpus callosum was markedly involved, with less extensive but rather uniform involvement of the basal ganglia and thalami. No region of the cerebrum appeared to be affected more than any other, nor was total lack of evidence of neoplastic activity noted anywhere. Scattered tumor cells were found within the mesencephalon, pons, and central nuclear masses of the cerebellum (Fig. 5*b*). Tumor cells were not found within the cerebellar hemispheres or the medulla, nor was there evidence of leptomeningeal extension in this region.

Comment

As did Nevin,[1] we found considerable variation in the form and shape of the individual cells constituting the process. He observed a wide range, from immature spongioblastic to mature astrocytic forms, but concluded that, despite this variation, there existed a definite tendency of the cells of the tumor to differentiate along normal lines. However, like others, he did not observe the mitotic figures that we found so consistently. One would suspect that regression to malignant glial forms would demand the presence of mitotic figures if current concepts of neoplastic growth are tenable.

Nevin considered that the prominence of mature forms along with immature forms was evidence against the neoplastic basis of the disorder. The apparent differences in involvement of the cortex and white matter in certain regions also were considered to defy the fundamental laws of neoplastic growth. The lesions in our cases showed all the features considered by Nevin; yet the progressive clinical course and microscopic findings suggested the presence of highly malignant gliomas.

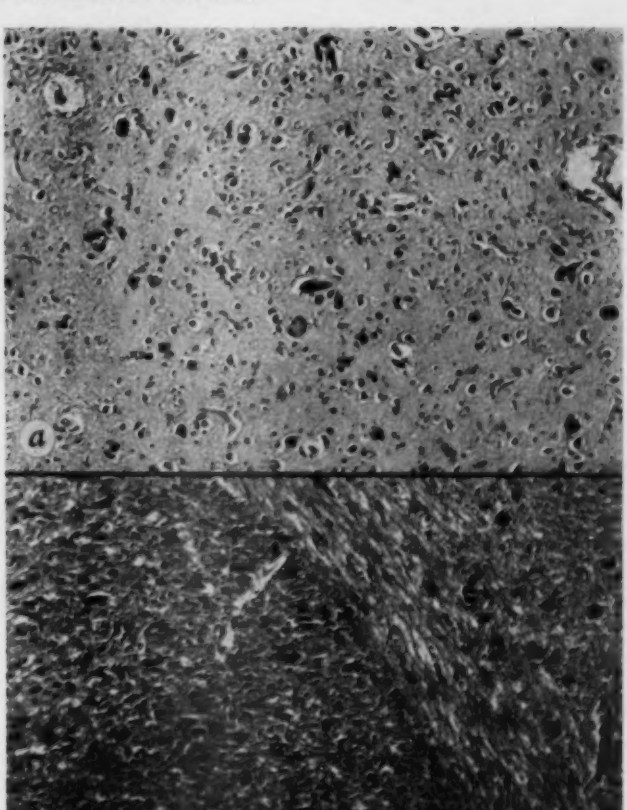

Fig. 5 (Case 2).—(*a*) Cortex showing extreme variation in form of multinucleated giant cells. Hematoxylin and eosin; × 100. (*b*) Pons. Similar extensive neoplastic invasion in the region of the brain stem was confined primarily to the zones occupied by long white-fiber tracts. Hematoxylin and eosin; × 110.

No single site of origin could be established in either of our cases. It would be difficult to conceive of this tumor spreading so rapidly and diffusely from a primary focus; rather, a process of widespread neoplastic transformation is suggested. It is our opinion that gliomas arise from preexisting cells by a process of dedifferentiation or anaplasia rather than from embryonic cell rests. The very presence of diverse degrees of dedifferentiation and anaplasia undoubtedly accounts for the variation and pleomorphism observed in these lesions. The forces producing widespread neoplastic change are unknown. Perhaps some "chemical" substance is responsible, as suggested by Weil.[20] We also consider the absence of "immature" neuronal elements in our cases as further evidence against the embryonic-rest origin of these tumors. Like Bailey and Beiser,[21] we found it difficult to identify immature neuronal elements and considered the nerve cells found in the midst of these gliomas as remnants of preexisting cells now located within the substance of the tumor.

That gliomatosis cerebri could arise from multicentric foci of origin must be considered. Scherer[22] found evidence of multicentric origin of gliomas in 10% of his series. Courville[23] found multicentric growth in 9.4% of examples of glioblastoma multiforme and in 6% of astrocytomas. In an extensive review of the problem of

multiple primary gliomas, he suggested that they might occur as (1) metastasis from a single growth, (2) a process of "discontinuous growth" whereby malignant gliomas are thought to "infect" local glial elements, resulting in malignant changes in them also, and (3) development of multiple foci of origin (thought by Courville to be the best explanation).

Leptomeningeal invasion by the neoplastic process is a striking feature and may account for part of the neoplastic spread by direct extension or perivascular invasion.[24] Leptomeningeal invasion was found in 7% of the astrocytomas observed by Elvidge and associates.[4] This factor merely emphasizes the difficulties encountered in any attempt to establish specifically how this particular group of cerebral neoplasms may attain such diffuse proportions. Like Nevin, we think that such extensive neoplastic changes could not plausibly have arisen and spread, leptomeningeally or otherwise, from a single point of origin.

Finally, there was no clinical evidence of any associated pathologic process in either of our cases that might suggest some relationship, as has been postulated so frequently in the past. We consider that the occasional reported occurrence of such tumors in Recklinghausen's disease does not prove their invariable relationship to this disease or any similar disorder. Morphologically, we cannot accept a possible origin for this disorder from Schwann's cells.

Summary

A study has been made of two cases of gliomatosis cerebri. The lesions in these cases were considered to be examples of neoplastic transformation of the malignant astrocytoma type.

A single site of origin of the tumor could not be established in either case. Such widespread tumors probably do not spread from a primary focus. Instead, a process of diffuse neoplastic transformation is suggested.

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Double Mitral Apparatus or Orifice

Report of a Case

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Double mitral apparatus is a congenital malformation of the left atrioventricular ostium. In this entity the mitral valve is replaced by two distinct orifices, both of which have separate valve leaflets, chordae tendineae, and papillary muscles (Fig. 1). This is a very rare congenital abnormality, as only 13 cases have been reported since 1876.[1-11] The following case study presents a typical example of this entity in combination with persistent ostium primum and ostium secundum, bicuspid aortic valve, and subacute bacterial endocarditis.

Report of Case

History

The patient was a 33-year-old woman who had had rheumatic fever at the age of 19. She developed increasing shortness of breath and ankle edema, for which she had been admitted to Meyer Memorial Hospital two months prior to the last entry. She was found to have inactive rheumatic fever, mitral stenosis, and congestive heart failure. Her response to digitalis and diuretics was satisfactory, but she refused further diagnostic studies and was discharged. The patient was symptomless in the interim.

The night before her second admission she became frightened after coughing up a cupful of blood and was admitted for completion of diagnostic studies.

Physical Examination

The patient was a well-developed, well-nourished white woman, in no acute distress. Vital signs were not remarkable. No enlargement of the heart was found. There were a soft diastolic murmur and a Grade 2 systolic murmur at the third interspace to the left of the sternum and an accentuated first sound at the apex. No evidence of congestive heart failure was found. The electrocardiogram revealed a right bundle-branch block, right ventricular hypertrophy, first-degree AV block, sinus rhythm, and nonspecific myocardial damage. Fluoroscopy showed a dilated pulmonary artery, with marked hilar dance, and pulmonary congestion. The aorta was hypoplastic; the left atrium was not enlarged.

Cardiac catheterization showed an atrial septal defect, through which the catheter was passed and led to a pulmonary vein, where the high pressure of 18/12 was found. The right ventricular output was 14.56 liters per minute.

These clinical and laboratory findings supported the following impressions: (1) interatrial septal defect with left-to-right shunt; (2) obstruction to outflow, as with mitral stenosis.

Surgery

The patient was operated on for correction of the mitral stenosis. At the operating table the aorta was observed to be half the normal size and the pulmonary artery twice as large. Two defects were felt in the interatrial septum. The mitral valve felt soft, and only minimal regurgitation was noted. The ostium of the valve was long and coned down to a 3 by 10 mm. opening and showed no calcification. This was fractured to admit two fingers.

After a satisfactory two-day postoperative course the patient died suddenly.

Autopsy Findings

Examination of the recently incised and sutured pericardium revealed a bloody effusion of 250 cc. Both visceral and parietal pericardium were covered with a fibrinous deposit.

On opening the right atrium, two interatrial septal defects were found, separated by a firm band of tissue 0.6 cm. in width and 3.8 cm. in length (Fig. 2). The upper defect (foramen secundum)[12] measured 4 by 3 cm. and was covered laterally by a fenestrated membrane. The lower defect (foramen primum) measured 2.4 by 5.6 cm.

A thickened tricuspid valve, 10 cm. in circumference with soft, red, opaque chordae, was next seen. A number of polypoid, edematous vegetations were present on this valve. The right ventricle was dilated, with a circumference of 12.5 cm., and the wall was hypertrophied, measuring 1.4 cm. below the AV ring. The pulmonary valve was widely dilated, with a circumference of 9.5

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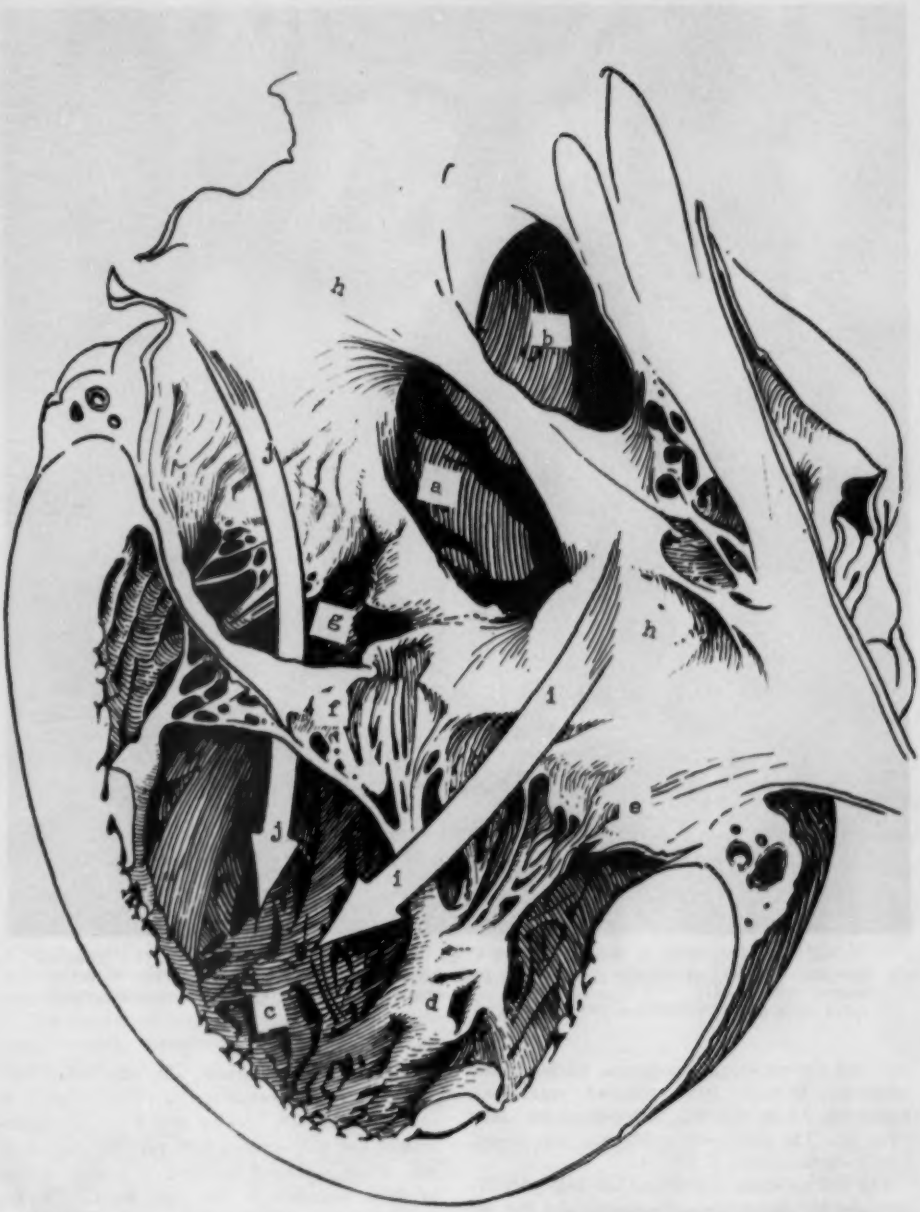

Fig. 1.—Appearance of the heart from left after reconstruction: (*a*) foramen primum; (*b*) foramen secundum; (*c*) left ventricular cavity; (*d*) posterior papillary muscle; (*e*) posterior leaflet of primary mitral apparatus, corresponding to normal mitral valve; (*f*) anterior leaflet of same valve; (*g*) supernumerary mitral orifice; (*h*) left auricular cavity; (*i*) arrow demonstrating normal atrioventricular orifice; (*j*) arrow demonstrating anterior (supernumerary) mitral orifice.

Fig. 2.—Appearance of the open heart from left: (*a*) foramen secundum; (*b*) foramen primum; (*c*) left ventricular cavity; (*d*) posterior papillary muscle; (*e*) posterior leaflet of normal mitral valve; (*f*) anterior leaflet of normal mitral valve; (*g*) supernumerary mitral valve, torn during valvulotomy.

cm., and the cusps were somewhat thickened and adherent. Multiple large polypoid vegetations, measuring 2.3 by 1.5 cm., occurred on the cusps (Fig. 3). The pulmonary artery was also dilated, with a circumference of 8.5 cm.

The left auricular appendage had been partially removed by the previous operation, and the remainder contained thrombi. The surgical suture line was imperfect, with one suture missing.

The mitral valve was examined by the finger before the right atrium was opened and was found to admit two fingers. Later it was realized that this was an extra valve, the so-called "supernumerary mitral valve." When the scissors cut through this orifice and laid open the left ventricle, a separate valve apparatus was seen which corresponded to the normal mitral valve. (Figs. 1, 5, and 6). This later opening was 8 cm. in circumference and was supplied with two thickened leaflets, which contained polypoid structures similar to those described in the right heart. Chordae tendineae were thickened and connected with posterior and anterior papillary muscles in a normal manner. The supernumerary mitral orifice after reconstruction measured 2 by 1.5 cm. in diameter. No leaflets were observed, although five chordae tendineae arose from the inferior lip of this orifice and attached to the posterior wall of the left ventricle (Fig. 5).

Fig. 3.—Appearance of pulmonary valve from right ventricular cavity: (*a*) markedly thickened posterior leaflet; (*b*) vegetations; (*c*) right ventricular cavity.

The left ventricle was not dilated, the wall being 1.2 cm. thick and 9.5 cm. in circumference.

The aortic valve had only two cusps, both of which were markedly thickened and adherent to each other. Vegetations were fewer here than at the other valves. A poorly marked raphe was present behind the anterior cusp. Both coronary arteries arose from the coronary sinus corresponding to the anterior cusp.

The lungs were hyperemic and edematous. There was complete atelectasis of the right and left lower lobes and part of the upper lobes. Pleural cavities contained 100 cc. of fluid bilaterally. A fibrin layer was found on the pleura.

The kidneys were of duplex type. The remainder of the autopsy findings were not remarkable except for the congestion of the viscera.

Microscopic Examination

The myocardium revealed generalized increase in connective tissue with evidence of slight chronic inflammation. The supernumerary orifice of the mitral valve showed distinct fibrosis and inflammation, as did the chordae tendineae attached to this valve apparatus. Slighter inflammatory and fibrotic changes were observed in the "normal" mitral valve, as well as in the remaining valves. Polypoid vegetations observed in the gross specimen (Figs. 3 and 5) were made of fibrous tissue and showed chronic, nonspecific inflammation. The rest of the organs showed hyperemia.

Comment

In the differential diagnosis of a left atrioventricular canal presenting two ostiums separately connecting the left atrium and the left ventricle, one must consider the following possibilities: (1) perforation of a valve leaflet due to inflammatory lesions; (2) perforated aneurysm of the leaflet [13,14]; (3) partial fusion of the leaflets due to secondary inflammation; (4) traumatic ruptures; (5) double mitral apparatus.

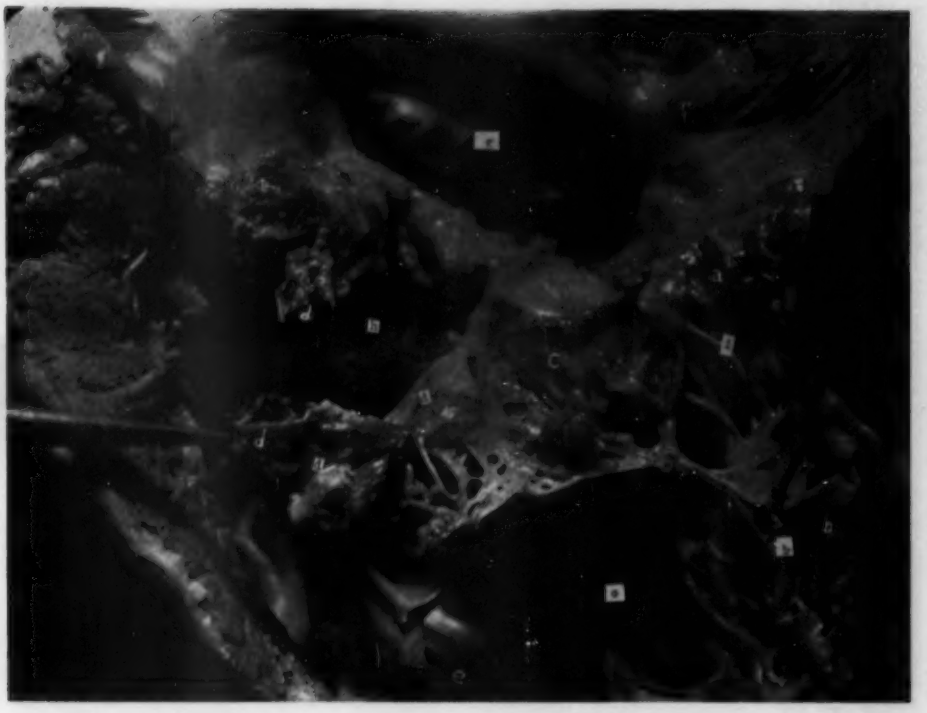

Fig. 4.—Appearance of the mitral area on a closer examination: (*a*) margin of posterior leaflet of mitral valve; (*b*) posterior papillary muscle; (*c*) torn edges of mitral valve (supernumerary mitral apparatus); (*d*) anterior leaflet of mitral valve; (*e*) left ventricular cavity; (*g*) foramen primum; (*h*) supernumerary mitral orifice; (*i*) chordae tendineae; (*f*) left auricular cavity.

The criterion previously applied to establish the diagnosis of double mitral apparatus is two separate orifices equipped with leaflets, chordae tendineae, and papillary muscles. Our case had a second ostium, definite chordae tendineae, and papillary muscles associated with it (Figs. 4 and 5), although the leaflets were absent. But the presence of a web between the two left chordae tendineae we consider sufficient evidence of congenital origin (Fig. 5).

Although at the time of autopsy this supernumerary mitral orifice was found to be torn, the site of operation was demonstrated and the lesion examined after reconstruction (Fig. 5). It was then found to be narrow to the digital examination, this explaining how the surgeon was misled in considering this as a mitral stenosis.

The question whether this case should be considered an example of ostium atrioventriculare commune was raised. Prevailing opinion of writers on the subject of ostium atrioventriculare commune require these conditions: interatrial septal defect, interventricular septal defect, undivided AV ostium, undivided common valves for entire canal, and free communication among four chambers of the heart.[18-21] Because our case did not demonstrate the last four conditions, complete form of ostium atrioventriculare commune was ruled out. We noted an entirely normal division of atrioventricular canal into a right and a left heart. The right atrioventricular orifice was furnished with a tricuspid valve, and the left atrioventricular canal contained a valve apparatus with two distinct ostiums. This description

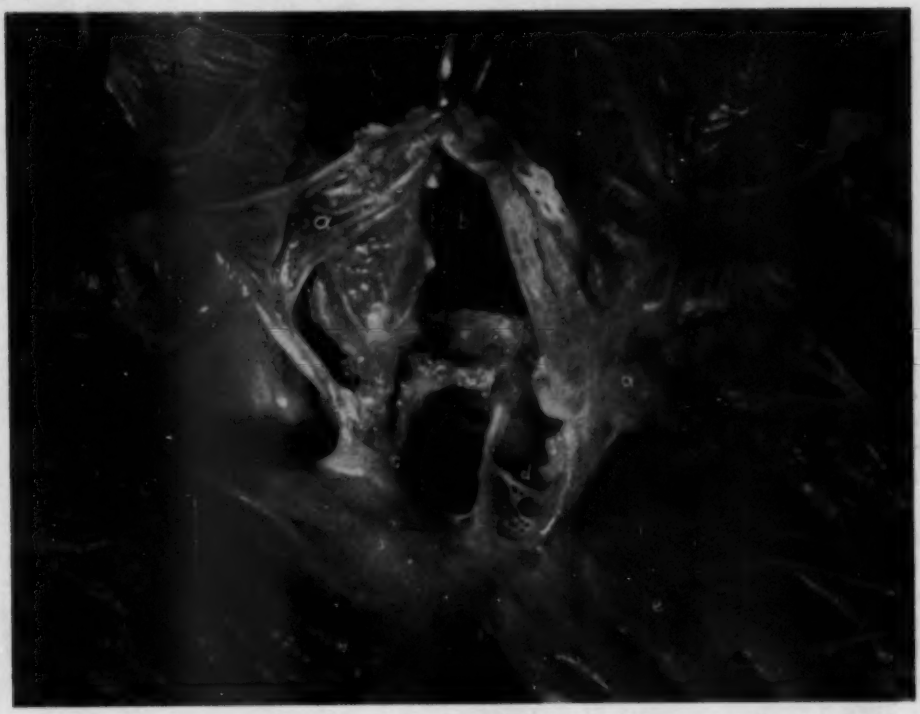

Fig. 5.—Appearance of anterior mitral leaflet from below after reconstruction: (*a*) ventricular surface of anterior mitral leaflet; (*b*) supernumerary mitral orifice; (*c*) papillary muscles of supernumerary mitral apparatus; (*d*) web between these papillary muscles; (*e*) left ventricular cavity.

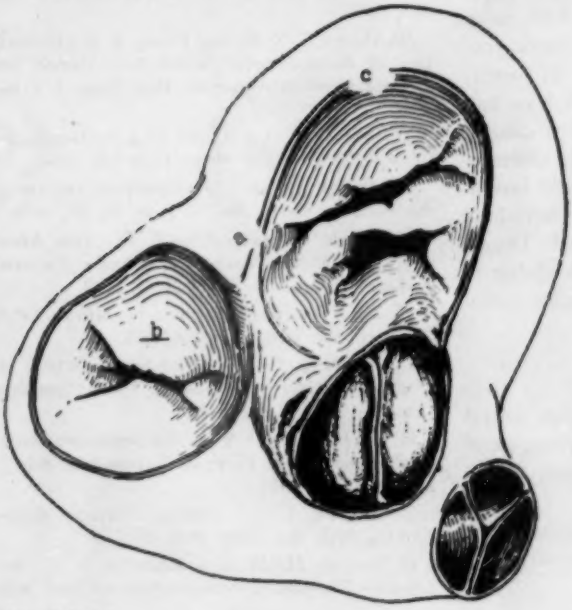

Fig. 6.—Appearance of heart as sectioned at level of atrioventricular groove: (*a*) right atrioventricular ring; (*b*) tricuspid valve; (*c*) left atrioventricular ring containing two separate valve apparatuses. The anterior orifice is the supernumerary orifice, which was felt at the operation and was cut at the autopsy table.

fulfils the criteria of partial persistent common atrioventricular canal of Wakai and Edwards.[28]

Septal defects have frequently been observed with other congenital cardiac diseases, such as ostium atrioventriculare commune; cleft mitral, pulmonary stenosis; double mitral apparatus, Lutembacher's syndrome, and atresia of the right ventricle. In many of these conditions development of the endocardial cushions is at fault.[12,15,22,27]

A bicuspid aortic valve was found which showed thickening suggesting a rheumatic etiology. In fact, all four valves revealed such inflammatory stigmata, as well as large vegetations, which are considered to be healed subacute bacterial endocarditis.[16,17]

It is generally felt that most bicuspid aortic valves occurring in an adult should be considered as congenital only when associated with other congenital malformations of the heart. This opinion and gross and histological findings in our case confirm the congenital nature.[25,26]

Since a complete summary of literature in this subject is given by Schraft and Lisa,[11] only the most prominent features will be discussed here: Double mitral apparatus was found to occur in a wide range of ages, from 16 to 71 years. Clinical conclusions are not possible because of paucity of details in reported cases. At least two patients were described as dying of congestive heart failure. In one of these antemortem diagnosis of mitral stenosis and insufficiency was made. In at least five cases loud precordial murmurs were recorded. Details of pathological examination may differ to some extent; however, all had double mitral orifices.

Summary

The 14th known case of double mitral apparatus is reported. Coexisting congenital and acquired lesions are described, and a brief discussion is given.

The surgical and diagnostic importance of this malformation is brought out.

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Ferrous Ion Uptake

A Specific Reaction of the Melanins

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In the course of unsuccessful attempts to produce colored nitrosotyrosine complexes in tissues with the divalent metals Hg^{++}, Cu^{++}, and Fe^{++},[7] a ferricyanide test was applied to see whether there had been any ionic Fe^{++} uptake in uncolored form. This resulted in a deep blue-green coloration of cutaneous melanin, with little or no color in any other tissue elements.

Experimental Study

Exploration revealed that the nitrite could be eliminated and that the original 24-hour exposure at 3 C could be shortened to 15-60 minutes at 25 C. Ferric sulfate could be substituted for ferrous sulfate with an apparently equal specificity of the coloration, but on substitution of ferrocyanide as the demonstration reagent melanin remained essentially unreactive.

At this point it appeared that the reaction might be similar in nature to the Golodetz-Unna ferric ferricyanide test and that the reduction of ferric to ferrous salt might be the essential factor. Melanin reduces ferric ferricyanide strongly.[5] To test the hypothesis that the reaction might depend on the presence of small amounts of ferric salt in the ferrous sulfate originally used, a quantity of ferrous chloride was prepared by reacting an excess of metallic iron (3 gm.) with 100 cc. of normal hydrochloric acid in a closed vessel from which the escaping hydrogen was bubbled out through a water trap. The resultant 1 N (0.5 M) ferrous chloride solution (pH 4.3) was boiled, filtered, made up to 100 cc. with small distilled water washes, and diluted 7.2 cc. to make 40 cc., thus approximating the 0.09 M concentration represented by 2.5% $FeSO_4.7H_2O$ (1 gm/40 cc.).

Melanin again colored dark blue-green with $K_3Fe(CN)_6$ after treatment with this freshly prepared $FeCl_2$.

Influence of Acid.—It was found that the reaction failed when the ferrous salt was adjusted to pH 1 and succeeded best at pH 3-5. The presence of even 0.5% acetic acid in the distilled water used to wash out the excess ferrous salt before reacting the section with ferricyanide seriously impaired the reaction. Sodium acetate (0.5%) and distilled water, four changes, five minutes each, were equally resisted.

Specificity.—In the course of the preliminary experiments, sections of mouse and guinea pig stomach, pancreas, duodenum, and colon; guinea pig heart, aorta, lung, and liver; rat trachea, esophagus, and thyroid; human midbrain, ocular and cutaneous melanomata, and scalp skin, as well as several blocks of guinea pig skin with both trichoxanthin [6] and melanin, were studied.

In the original nitrosation experiment a weak to moderate reaction of mouse gastric and guinea pig duodenal enterochromaffin was observed. This disappeared when $NaNO_2$ was omitted from the ferrous sulfate solution. A pale blue or green coloration of the keratinization zone of guinea pig hair cortex was observed in one block. Otherwise, there were only faint greenish-

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yellow colorations or completely unstained tissue, except for melanin.

Melanin reacted strongly blue-green in Langerhans cells and hair matrix cells of guinea pig skin, colored greener in hair medulla melanins, and remained brown in hair cortex and outer stratum corneum. In the human eye, part of the pigment in the degenerated retinal pigment epithelium colored only greenish yellow; that in the melanoma, choroid, and iris and ciliary body chromatophores and epithelia colored dark blue-green to green. The human skin and cutaneous melanoma pigments colored dark green to blue-green. The yellow-brown pigments human neuromelanin and guinea pig trichoxanthin also colored dark green to dark green-blue.

At this point human autopsy and surgical material containing most of the commoner lipofuscin pigments, hemosiderin, and spinal ganglion pigment was added to the series: brain (thalamus, midbrain, anterior pons with locus ceruleus, medulla at olivary nuclei, spinal cord, and ganglia); heart; liver; testis; epididymis; seminal vesicle, with both epithelial and muscle pigment; ovary, with hemosiderin, hematoidin, and lipofuscin pigments; adrenal; lung, with hemosiderin and carbon; spleen from cases of congenital hemolytic anemia and generalized siderosis, and appendices with moderate melanosis. Guinea pig intestine, with the homologous "enterosiderin"[3] pigment in villi and cecal mucosa; rat liver, with experimental cirrhosis and ceroid pigment, and rat uterus from α-tocopherol deficiency, with copious ceroid, were included for comparison.

Control sections were stained with Nile blue sulfate to demonstrate lipofuscin,[4] with diamine silver (15 minutes) for melanin, or with potassium ferrocyanide-acetic acid mixture for hemosiderin, to demonstrate the presence and amount of the pigment. Sections from all blocks were also subjected to two additional controls: an incubation for 1 hour in 1.25% Na_2SO_4 in place of 2.5% $FeSO_4.7H_2O$, before the 30-minute ferricyanide bath, and to a direct ferricyanide reaction.

As before, melanin in neurons of the vagus nucleus, locus ceruleus, and substantia nigra reacted strongly. Lipofuscin in the nuclei thalami anterioris, medialis, and lateralis; nucleus subthalamicus; nucleus dorsalis raphies, and nucleus reticularis tegmenti pontis; nucleus olivaris inferior; nuclei hypoglossus, vagi and vestibularis spinalis, and the anterior horn cells of the spinal cord and spinal root ganglia colored at most pale greenish-yellow. The so-called melanin of the spinal ganglion cells colored at most yellowish-green.

Lipofuscin in the epithelium and muscle of the seminal vesicle colored light greenish-yellow; the lipofuscins in the Leydig and germinal epithelial cells of the testis and of the epithelium of the epididymis, the epinuclear pigment of the heart, and the adrenal and hepatic lipofuscins failed to color appreciably.

In one ovary phagocytes contained both hemosiderin and lipofuscin, and in many phagocytes the ferrous ion uptake reaction revealed numbers of isolated dark green granules among the predominant, often larger, pale yellow ones. However, this staining was also given by the Na_2SO_4 and direct ferricyanide controls; hence this is to be recognized as one of the rare examples of ferrous-iron-containing pigment. In several other ovaries where both hemosiderin and lipofuscin were present, no ferrous ion was demonstrable, and the ferrous ion uptake reaction was negative. Dark brown amorphous and crystalline hematoidin failed to react. Formaldehyde pigment in blood vessel contents failed to react. Hemosiderin also failed to react in spleen and pulmonary phagocytes, though strongly iron-positive by the usual ferrocyanide test.

Ceroid pigments of the rat liver and uterus, human melanosis coli pigment, and guinea pig enterosiderin all failed to react.

Counterstains.—Red and brown basic dyes, even at pH 2, change the color of the melanins to black. Fuchsin gives a quite good red nuclear stain at 1:1000 in pH 2 M/10 HCl+KH_2PO_4 buffer. Safranine is equally sharp, but gives brownish-red crystalline precipitates, as with other ferricyanide techniques. Excellent contrast of dark green pigments with yellow to brown cytoplasm and red collagen was attained by interposing a five-minute Van Gieson stain after completion of the ferricyanide step.

Influence of Fixations.—Chromate-containing fixatives with mercury (Spuler's and Zenker's fluids) or without the mercury (Kose's bichromate-formalin) inhibited the reaction. Alcoholic fixatives: acetic acid-alcohol-formalin (5:85:10), 75% alcohol, Carnoy's acetic acid-alcohol-chloroform (10:60:30), chloroform-methanol (50:50, two days at 60 C), and heavy metal: 8% $HgCl_2$ or $Pb(NO_3)_2$ in 75% alcohol gave the same positive results as neutral aqueous formaldehyde solutions.

Blocking Reactions.—The reaction of cutaneous melanin is unaffected by prior exposure to the following reagents: 5% $HgCl_2$, 16 hours, 25 C; 10% iodine in methanol, 16 hours, 3 C; 5% $Na_2S_2O_4$, 1 hour, 25 C; pyridine 24 hours, 60 C; 0.1 N HCl in methanol, 4 days, 60 C; methanol, 4 days, 60 C; 1 N HCl (aqueous), 1 hour, 60 C; 10% aniline in glacial acetic acid, 1 hour, 25 C; glacial acetic acid 1 hour, 25 C; distilled water 24 hours, 60 C; 40% acetic anhydride in pyridine 18 hours, 25 C.

Exposure to 5% $K_2Cr_2O_7$, two days, 25 C, completely prevented the reaction of cutaneous melanin, guinea pig trichoxanthin, and human neuromelanin. Hydrosulfite reduction, followed by acetylation as above, also prevented the reaction of cutaneous melanin and of human neuromelanin.

The reaction of neuromelanin was also much weakened by the iodination procedure and by direct acetylation.

As previously reported, methylation removes trichoxanthin, and in this series acetylation also dissolved it.

Appropriate controls were used in each instance to demonstrate the effectiveness of the blockade reaction: $HgCl_2$, inhibition of the ferric ferricyanide SH reaction of basal hair cortex, iodination and chromation abolition of the ferric ferricyanide reaction of skin melanin, aniline and hot distilled water blockade of the Schiff aldehyde reaction when interposed after periodate oxidation, acetylation inhibition of the periodic acid-Schiff reaction, and methylation abolition of basophilia. Pyridine, methanol, and glacial acetic acid served as solvent controls on the blocking reactions.

Comment

The blocking reactions appear to indicate that the prosthetic group or groups in melanin responsible for the Fe^{++} uptake are not carboxylic acid, aldehyde, hydroxyl, or amine. The inhibition of uptake at low pH levels seems to rule out sulfuric and phosphoric acid residues.

The response to oxidation and to hydrosulfite reduction followed by acetylation is the same as that with the metal reduction reactions. I have assigned this behavior elsewhere [5] to the presence of *o*-quinhydrone groupings.

Feigl [9] recounts formation of inner complex metal salts with organic compounds possessing adjacent hydroxyl and keto groupings, such as rhodizonic acid and tetrahydroxyhydroquinone, as well as describing a number of iron catechol complexes.[10]

Considering that the semiquinone radical or monomer of *o*-quinhydrone [11] should present adjacent keto and hydroxyl groups, it would appear that this structure might well account for the observed binding of ferrous ion. The catechol structure produced on reduction of melanin by hydrosulfite should also be capable of binding Fe^{++}. This accords with the noninhibition of the reaction by reduction.

Relation to Ferric Ferricyanide Reaction of Golodetz and Unna[8]

While preliminary treatment of melanin with ferric chloride solution results in a positive ferricyanide reaction of melanin, this is not true of other reducing sites, such as enterochromaffin cells, although ferric chloride does oxidize this substance to the point of not reducing ferric ferricyanide. Further, the iron reaction demonstrated is for Fe^{++}, not Fe^{+++}. It has also been demonstrated[6] that ferric chloride oxidizes melanin, and the present work demonstrates that it is reduced to the ferrous form in the process. Nevertheless, the ferrous ion uptake reaction appears to depend on the same *o*-quinhydrone configuration that seems to be responsible for the metal reduction reactions.

Recommended Technique

All chromate fixatives should be avoided. Other fixatives—mercurial, formaldehyde, alcoholic, etc. —are well tolerated.

1. Paraffin sections: Deparaffinize and hydrate as usual.
2. Immerse for one hour in 2.5% ferrous sulfate ($FeSO_4.7H_2O$)
3. Wash 20 minutes in distilled water (four changes).
4. Immerse 30 minutes in 1% potassium ferricyanide ($K_3Fe(CN)_6$) in 1% acetic acid.
5. Wash in 1% acetic acid.
6. If desired, counterstain 5 minutes in Van Gieson's trinitrophenol (pictric acid) acid fuchsin mixture (100 mg. of acid fuchsin: 100 cc. of saturated aqueous trinitrophenol solution). Do not use hematoxylin.
7. Dehydrate in two changes each of 95% and 100% alcohol; clear in alcohol-xylene (50:50) and two changes of xylene.
8. Mount in synthetic resin. If not counterstained, use cellulose caprate. Results: melanin, dark green; background, faint greenish or unstained. With Van Gieson stain: collagen usual red, muscle and cytoplasm yellow and brown.

Summary

Exposure of melanin and melanoid pigments first to a solution of a ferrous (or ferric) salt and then to a potassium ferricyanide solution results in a highly selective Turnbull's blue coloration, which, by virtue of the native brown color, results in a dark green.

This reaction is highly selective. No other pigments react, except for the rare example of hemosiderin, which reacts directly to ferricyanide. Other tissues remain completely uncolored or color faint green.

The reaction is probably due to formation of an inner complex ferrous salt, which then reacts with ferricyanide to form Turnbull's blue.

A Van Gieson counterstain may be performed, with excellent contrasts.

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health (14).

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Case Reports

Congenital Dilatation of Pulmonary Lymphatics

LEONARD MAIDMAN, M.D., and ROY N. BARNETT, M.D., Norwalk, Conn.

We present the following case because of its rarity, only three previous cases being known, the relatively long duration of life (28 days), and the associated right ventricular hypertrophy.

Report of Case

The patient was born of a 24-year-old white woman, bipara, tertigravida, on March 7, 1954. Siblings born in 1948 and 1950 were well. Gestation was uneventful and delivery spontaneous, without difficulty. Birth weight was 7 lb. 13 oz. (3544 gm.). The child appeared healthy but had some mild episodes of cyanosis during the first four days of life. She was fed on an evaporated milk-Dextri-Maltose formula but never ate well or gained any weight. On March 20 there was the onset of irritability, cough, frequent crying, and cyanosis on crying. Other members of the family had respiratory infections at this time. On March 31 she had mild pharyngitis, which improved after two doses of procaine penicillin G, U. S. P., 200,000 units each. On April 4 she suddenly became unconscious, had severe respiratory distress, and died.

Gross Autopsy Findings

The body was fairly well developed and nourished and weighed 7 lb. 9 oz. (3430 gm.). The heart was not enlarged, weighing 26 gm. (expected weight 20 gm.). The right ventricle was definitely hypertrophied. No other unusual features were present. The lungs were large, heavy, and wet, weighing 127 gm. together. They were diffusely subcrepitant, with no areas of consolidation. No cysts were noted. The liver was congested and weighed 205 gm. (expected weight 127 gm.).

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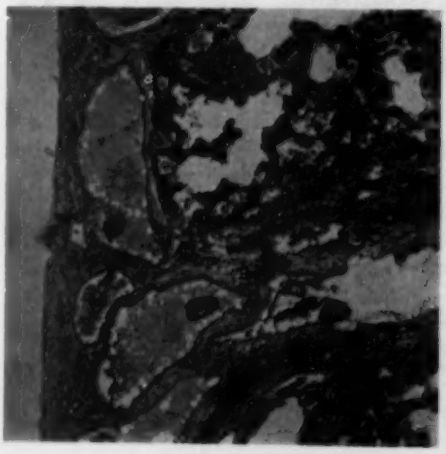
From the Medical and Laboratory Services, Norwalk Hospital. Attending Physician, Department of Medicine, Norwalk Hospital (Dr. Maidman); Associate Clinical Professor of Pathology, Yale University School of Medicine (Dr. Barnett).

Microscopic Autopsy Findings

The left ventricular muscle fibers were intact. The right ventricular fibers were enlarged to at least twice the size of the ventricular fibers on the left. Many had large, bizarre nuclei; others had a clear cytoplasm. In the lungs there were numerous dilated, irregularly shaped lymphatic vessels, located in the subpleural tissues (Fig. 1), about the arteries and bronchi (Fig. 2), and in the septa. They varied from a few micra to 5 mm. in diameter. They had thicker walls than normal lymphatics. They were distinguished from veins by the presence of valves (Fig. 3), the absence of erythrocytes, and the presence of pink-staining amorphous material and a few lymphocytes. The alveolar walls were generally thick and slightly congested. Almost no thin alveolar septa of the type expected at this age were found. The air spaces contained pink-staining amorphous material, macro-

Fig. 1.—Dilated subpleural lymphatics containing lymph. Reduced to 80% of mag. × 110.



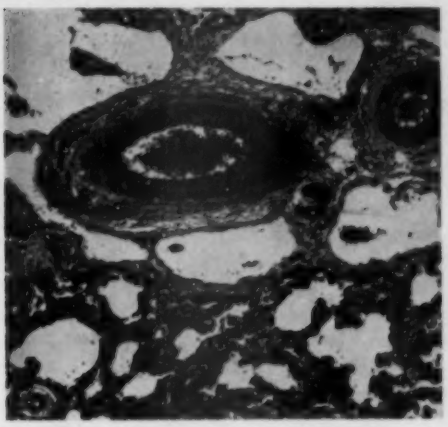


Fig. 2.—Dilated perivascular lymphatics. Reduced to 80% of mag. × 110.

phages, and in some areas many erythrocytes. The liver showed slight acute congestion. Extramedullary hemopoiesis was marked in the spleen, moderate in the liver, slight in the lungs, and not found elsewhere.

Comment

The entity has been well defined by Giammalvo.[1] He cites only two earlier cases, that of Virchow (1862) in a 9-month-old cretinoid male fetus and that of Klebs (1889) in a 6-month-old fetus. Giammalvo's patient was a female infant who lived 1 hour and 44 minutes. She had multiple pulmonary blebs, ranging up to 0.6 mm., occurring in unexpanded lungs. Histologically these were dilated lymphatics identical with those in our case.

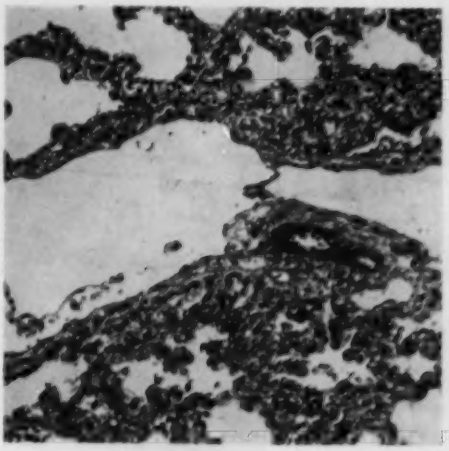
Inasmuch as the dilated pulmonary lymphatics described are congenital defects, it is relevant to mention that the pulmonary lymphatics in fetal life are relatively large.[2] The dilated lymphatics in the present case may well represent a persistence of these vessels beyond the usual period, though this hypothesis does not explain why they have relatively thick walls. Several alternative explanations merit discussion. First, could there be an obstruction of major pulmonary lymphatics? Against this hypothesis is the absence of chylothorax or pulmonary lymphorrhagia.[3] Second, could there be pulmonary venous obstruction with compensatory lymphatic dilatation? This seems unlikely, because no such obstruction was found in the gross dissection of the pulmonary veins, and because there was no histologic evidence of severe chronic venous and pulmonary congestion.

The right ventricular hypertrophy could result from stiffening of the lungs as a whole by the dilated lymphatics, with consequent loss of normal pumping action due to respiratory excursion, thereby increasing the work of the right ventricle. Alternately, the hypertrophy could result from increased resistance by the thickened, poorly developed alveolar walls, or it may have other, unknown causation. Certainly, the absence of chronic passive congestion of the liver indicates that chronic right heart failure was not present. On the other hand, the extramedullary hemopoiesis and clinical cyanosis indicate chronic anoxia which was primarily pulmonary, rather than cardiac, in origin.

Summary

A case of congenital dilatation of the pulmonary lymphatics is presented. The child lived 28 days and died with cyanosis and right ventricular hypertrophy. Only

Fig. 3.—Valve projecting into lumen of large lymphatics. Reduced to 80% of mag. × 470.



three previous cases are known, and none of these patients lived over two hours.*

* At least three additional cases of congenital lymphatic dilatation have been found in the files of the Armed Forces Institute of Pathology.

Medical and Laboratory Services, Norwalk Hospital.

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Epithelial Cyst of the Heart

FREDERICK C. MARSHALL, M.D., Montreal, Canada

Epithelial cysts of the heart are extremely rare, and this, together with the remote possibility that such a cyst might be encountered during heart surgery, justifies report of an additional case.

Report of Case

Clinical History

A 26-year-old white man developed diabetes insipidus and symptoms and signs of mild hypopituitarism. Three years after the onset of symptoms, a space-occupying suprasellar lesion was diagnosed at craniotomy as a glioma of the optic chiasm. Removal of the tumor was not attempted, but the patient was given radiotherapy. His neurological signs and symptoms progressed, and he died in coma one year later.

Pathological Findings

The heart weighed 245 gm. A small, tense, translucent, egg-shaped cyst, measuring 5×4×4 mm., arose by a short fibrous stalk from the posterior wall of the left ventricle. It took origin about 2 cm. from the mitral valve ring, being attached between two trabeculae carneae near the base of the main posterior papillary muscle. The external surface of the cyst was covered with a single layer of endothelium. Its pedicle and wall were composed of strands of collagenous tissue (Fig. 1) with a small amount of muscle. It was not ascertained whether the muscle was of smooth or of cardiac type. The internal surface of the cyst was lined with a single layer of cuboidal or columnar ciliated cells (Fig. 2). The cyst contained gelatinous material, in which were occasional large round cells with vacuolated cytoplasm. Best's mucicarmine (carmine-aluminum chloride) stain for mucin was negative. There was a minimal degree of subendocardial fibrosis in the region of the cyst. No other cardiac anomaly was noted.

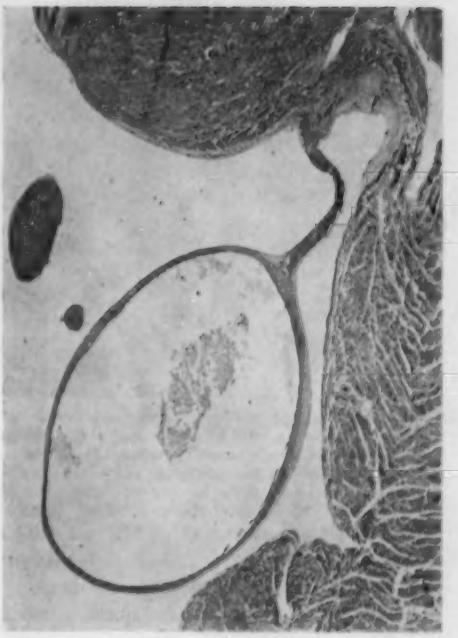

Fig. 1.—Cross section of the cyst, showing the narrow stalk and the relation of the cyst to the adjacent myocardium. A small amount of protein coagulum lies within the cyst, and two transversely sectioned chordae tendineae are seen near the cyst. Hematoxylin-eosin stain; × 13.

The brain weighed 1605 gm. Coronal sections revealed four separate tumors, varying from 0.7 to 2.5 cm. in greatest diameter, and located in the hypothalamus, the head of the caudate nucleus, the left occipitoparietal region, and the pons. The hypothalamic tumor had infiltrated the optic

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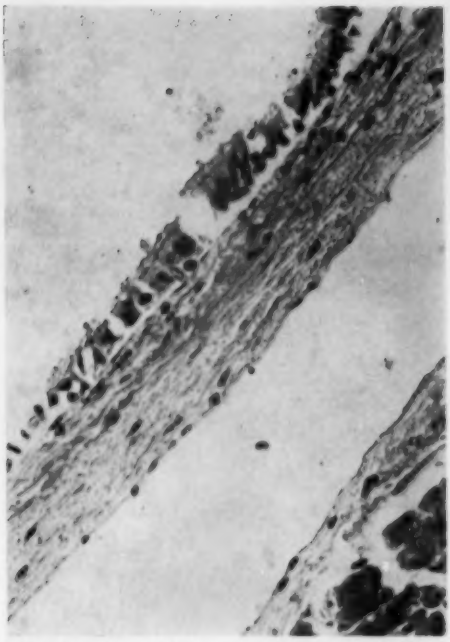

Fig. 2.—Wall of the cyst, composed chiefly of strands of fibrous tissue. The isolated, darker-staining elements are muscle cells. The cyst is lined internally by a single layer of columnar ciliated epithelium, and it is covered externally by endothelium. Hematin-phloxine-saffron; reduced approximately 2/5 from mag. × 400.

chiasm and the proximal parts of the optic nerves and had extended superiorly to block the third ventricle. The lateral ventricles were slightly dilated. The tumors were all well differentiated astrocytomas, but they differed considerably in details of cytology and morphology. No neoplastic bridges were found connecting the tumors with one another. The spinal cord and peripheral nerves were unremarkable.

The body was moderately obese with a feminine distribution of hair and fat. The external genitalia were small. The pituitary gland was normal in size and shape, but the anterior lobe was slightly edematous, with some atrophy of cells adjacent to the dorsum sellae, and the posterior lobe was atrophic and fibrous. The adrenals showed cortical atrophy, weighing 4.5 and 5.5 gm. The prostate gland was atrophic. The testes showed few Leydig cells and no spermato-

Epithelial Cysts of the Heart—Tabular Summary of Literature

Ref. No.	Sex	Age	Cause of Death	Location of Cyst	Size of Cyst	Lining Epithelium	Additional Data
1	M	45 yr.	Pneumonia (Ca stomach)	L. ventricle — posterior papillary muscle	4 mm. diameter	One to two layers, flat to columnar ciliated	Diverticula among muscle bundles
2	M	45 yr.	Echinococcosis	L. ventricle — anterior wall near papillary muscle	21X18X17 mm.	Columnar ciliated	Two smaller (4 mm.) cysts
3	F	Newborn	Meningoencephalocele	IA septum, above tricuspid valve	Lentil-sized	Squamous & columnar	Two small cysts and sweat-gland-like structures in wall
4	F	71 yr.	Ca of stomach; subdural hemorrhage	IA septum, bulging into R. atrium	Multiple, small	One to two layers, cuboidal to columnar	Caused heart block once (?)
5	F	64 yr.	Ca of cecum; peritonitis	L. ventricle — posterior papillary muscle	10X4X3 mm.	One to two layers, ciliated	Gelatinous content
6	F	51 yr.	Ca of colon; pulmonary embolus	L. ventricle — posterior wall, near AV sulcus	Cherry-sized	One layer, columnar ciliated	----
7	M	28 yr.	Miliary tuberculosis	L. ventricle — within wall	5.5X5X6 mm.	One layer, cuboidal ciliated	----
8	M	44 yr.	Myocardial infarct	L. ventricle — center of lateral wall	9 mm. diameter	Columnar & cuboidal, ciliated	Gelatinous content
9	F	29 yr.	Rheumatic heart disease	Posterior wall L. atrium and AV sulcus	Multiple, small	Cuboidal	Cellular debris in cyst
10	F	58 yr.	Cerebral embolus	L. ventricle — lateral wall, below AV sulcus	16X10X9 mm.	Columnar ciliated	Cartilage, elastic tissue, & gland-like structures in wall
11	F	6 mo.	Pneumonia; congenital heart disease	Interatrial septum	2.5X2X1.5 cm.	Stratified squamous	Multiple small cysts
12	F	73 yr.	Mitral insufficiency	L. ventricle—posterior wall	Cherry-sized	Pseudostratified, ciliated	Basal epithelial cells flattened
Author	M	30 yr.	Multiple brain tumors	L. ventricle—posterior wall, pedunculated	5X4X4 mm.	One layer, columnar ciliated	Gelatinous content

genesis. An acute tracheobronchitis was present. The lungs were congested and edematous, weighing 620 and 610 gm., and showed early bronchopneumonia.

Comment

The accompanying Table summarizes the 12 cases of epithelial cyst of the heart reported.[1-12] The present case is the 13th. In every case the cyst of the heart was discovered incidentally at autopsy.

Epithelial cysts of the heart can be divided into two types: those lined with ciliated and those lined with nonciliated epithelium. Of the 13 cases in the Table, 9 were of ciliated cysts and 4 of nonciliated cysts. The two types differ as to site of origin and number. The ciliated cysts arise in the left ventricle; the nonciliated, in the atria. The ciliated cysts are usually solitary; the nonciliated, multiple.

It is unlikely that the two types of cysts have the same origin. Five theories have been proposed to explain their occurrence: (1) sequestration of respiratory epithelium in the heart at an early stage of fetal development[6,12]; (2) dystopia or anomalous differentiation of cardiac mesoderm[10]; (3) displacement of thyroid or postbranchial body tissue[4,11]; (4) malformation of the epicardium (nonciliated cysts only)[2]; (5) origin in dermoid cysts (squamous-cell cysts only).[3] The sequestration of respiratory epithelium in early fetal life seems best able to explain ciliated cysts similar to the one reported here. There is a stage in the development of the embryo when respiratory epithelium could easily be included in the heart,[8,12] and the ciliated cysts of the heart are similar to mediastinal and esophageal cysts, thought to take origin from respiratory epithelium.[5]

The cyst in the present case resembled the ciliated cysts reported previously in that it was solitary and occurred in the wall of the left ventricle, but it was unique in being pedunculated. The presence of muscle elements in all parts of the wall and stalk suggests the cyst lay within the myocardium at one stage of its development. The pedicle may have been formed during enlargement of the cyst or during the progressive differentiation of the endocardium and myocardium. It is possible that it lay at one time in a trabecula carnea or a papillary muscle, which was destroyed as the cyst enlarged.

In addition to the cyst of the heart, the patient had multiple, well-differentiated astrocytomas of the brain. Multiple gliomas of the brain are rare except in cases of glioblastoma multiforme or von Recklinghausen's disease, and neither of these conditions was present in this case. The gliomas of the brain and the cyst of the heart may have been manifestations of the same dysontogenetic process, but, as in no other case of cyst of the heart have tumors of the brain been present, the coexistence of the two conditions was probably fortuitous.

Summary

A case of epithelial cyst of the heart is reported. The cyst was pedunculated, lay within the left ventricle, and was lined with ciliated epithelium. It was discovered incidentally in a 30-year-old man who died of multiple gliomas of the brain. The 12 cases of heart cyst previously reported are summarized, and the main theories of origin of the cysts are listed.

Dr. J. Olszewski, of the Montreal Neurological Institute, gave assistance in the neuropathological aspects of this case.

Montreal General Hospital.

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Obituaries

GEORGE LYMAN DUFF, M.D.
1904-1956

G. Lyman Duff was born on Jan. 26, 1904, in Hamilton, Ont., Canada, the son of Charles and Elizabeth Ann Duff. His schooling was in Hamilton, and in 1922 he began his university education at Victoria College, University of Toronto. During the summer vacations he studied in the Government Marine Biology Laboratories at St. Andrew's, New Brunswick. It was there that his first scientific investigation was made. It was a happy initiation to research that left pleasant memories for later years and gave material both for a master's thesis in biology and his first scientific publication, on "Factors Involved in the Production of Annual Zones in the Scales of the Cod." The degree of Doctor of Medicine was obtained from the University of Toronto, in 1929, together with a prize in psychology and psychiatry, and a second publication appeared in the *Toronto University Medical Journal.*

It was Lyman Duff's original intention to enter clinical medicine, and, in partial preparation for this, he entered pathology at the University of Toronto. It became his career. The year 1931 found him at Johns Hopkins' in the depart-

GEORGE LYMAN DUFF, M.D.
1904-1956

ment of pathology as a Fellow of the National Research Council (U. S.), and 1932 saw the award of the degree of doctor of philosophy by the University of Toronto for a thesis on "Experimental Studies upon Arteriosclerosis." In 1935 he returned to Toronto from Baltimore and married Isobel Farrell Griffiths. The Duffs came to Montreal and McGill University in 1939, where he was

appointed to the chair of the Strathcona Professor of Pathology, which had become vacant with the retirement of Prof. Horst Oertel. He retained the chair and directorship of the Pathological Institute at McGill until his death, on Nov. 1, 1956, and to these duties he added those of dean of the Faculty of Medicine from 1949 until the summer of 1956.

Lyman Duff's scientific and administrative abilities were widely recognized. He was consulting pathologist to some eight hospitals and a past president of the American Association of Pathologists and Bacteriologists, the International Academy of Pathology, the Quebec Association of Pathologists, and the American Society for the Study of Arteriosclerosis. He served on the editorial boards of five American and one Canadian medical publications and was an examiner for the Medical Council of Canada and for the Royal College of Physicians and Surgeons of Canada. He served the Medical Research Division of the National Research Council of Canada and the Canadian Cancer Society. He was president of the National Cancer Institute of Canada at the time of his death. He was a Fellow of the Royal Society of Canada and was awarded its Flevelle Medal in 1956. There were few facets of medical education, science, or administration in Canada to which he did not contribute in some way.

This much for the formal aspects of his biography. To those who were his colleagues there are other important things to remember. Lyman Duff was an attractive personality. When one thinks of him, there first comes to mind his love for and enjoyment in his family, then one remembers him as a teacher, and then one recalls his obvious abilities as a scientist, a pathologist, and an administrator. His family was his constant interest. His pride in his wife and their four children was as agreeable as it was well founded. Each week brought happy mutual adventures; their holidays were always shared in the north country, and the children were an integral part of the family's social life. One recalls an evening when one of the small children sat on father's knee and, because it lay close to hand, "Gray's Anatomy" was explored like "Mother Goose," producing an illustrated bed-time story of considerable merit and enormous enjoyment. Even in the later, busy years, he never allowed other commitments to deprive him of his happy appointments with his family.

As a teacher he was excellent. Teaching was, I believe, his chief professional interest. His lectures were not particularly exciting, for his style seemed somewhat plodding. Nevertheless, the student soon became aware that here was a teacher of great clarity of thought who could fuse the teaching of principles and of particulars into a valuable whole. His approach to the teaching of pathology was that of both the practical clinician and the biological scientist. No lecture was without its practical content, but equally, no lecture failed to indicate where unsolved problems lay. Each lecture was a lesson in how to think. To the students he was able simply to convey the values of humanity and humility. The pathology courses at McGill extend through both the second and the third years of the medical course. At one time Lyman Duff gave more than half of them himself, but with increasing responsibilities he was obliged to limit himself to the lectures in general pathology, for he regarded them as the most useful aspect of pathology for the student. Latterly, his duties restricted him to conducting with tact and humor an informal conference with the students in which together they reconstructed a clinical case from the postmortem findings. These were sufficiently popular that it was not unusual to find former graduates who were

visiting in the city sitting in attendance among the students. He possessed the rare tact of knowing when to speak from his large knowledge of pathology and, even more, of knowing when to remain silent and allow the students to speak. Lyman Duff was a students' professor and dean. It is, perhaps, the most telling comment on their admiration for him to find that the student body has spontaneously organized and is financing an annual memorial lecture in his name. So far as I am aware, this is the first time the student body has done this honor to any faculty member since the medical school held its first session, 124 years ago.

To his postgraduate students "The Chief" was an equally tactful master. Following the restricted activities of the war years his department graduated many students with the degree of M.Sc. or with the degree of Ph.D. in pathology. It trained numerous hospital pathologists and provided two professors to lead departments at other universities. His view of postgraduate training was a mature one. Very simply, he expected each member to do his best. Departmental relationships were informal, and, as long as duties were performed, hours of work were optional. He wanted what he called a "happy lab" and was careful to select his staff accordingly. Discipline was seldom exercised unless there occurred a breach of manners or of function. In his hands a disciplinary lecture was potent. He regarded experimental training as extremely useful if not essential to all divisions of pathology and went to some lengths to stimulate research of a sort that would not only contribute something to medical knowledge but that would also be a suitable vehicle for teaching the scientific method. To him, the man was more important than the problem, and one had the sense of being a colleague rather than a neophyte. It was the most educational of experiences to sit with him to discuss an experimental project or write a paper, for he had a happy, almost intuitive ability to reach the core of a problem. It was, perhaps, his relaxed enjoyment and pleasure in research together with a genuine liking of people that made his association with his staff so agreeable. Independence of thought and action was a right he gave to his junior staff as soon as they would accept it.

It is natural to look back from Lyman Duff's life as a teacher and experimental pathologist to those who were his teachers. Before he came to McGill he had been guided by such pathologists as Oskar Klotz, W. G. MacCallum, Arnold Rich, W. L. Robinson, and William Boyd. Each of them contributed to his training and experience, but perhaps the most influential was Klotz, for it was he who gave Duff the experimental approach to pathology and who initiated his interest in arteriosclerosis. At the time Lyman Duff began to study pathology with him at Toronto, Oskar Klotz was at the height of his reputation and was shortly to culminate a lifetime of research on arteriosclerosis with an important review (Proceedings of the Second Congress of the International Society for Geographic Pathology, Utrecht, 1934). With Klotz, Duff's innate analytical and inductive abilities flowered. He enjoyed, as he put it, "just finding out how things worked." The result of their association was a Ph.D. thesis of unusual value on experimental arteriosclerosis and a life-long preoccupation for Duff with experimental pathology and especially with arteriosclerosis. This predilection for experimental pathology and arteriosclerosis can be traced beyond Oskar Klotz and back to McGill University. Klotz was introduced into these subjects by the then Strathcona professor of pathology at McGill, George Adami, who had brought from his teacher Roy, at Cambridge, an interest in experimental

method and cardiovascular pathology. Klotz began to study pathology with Adami in 1903. Maude Abbott was a member of the department. The times and the teachers were right, and the interest in experimental cardiovascular disease grew in Klotz's hands to be returned to McGill again when Lyman Duff came to the Strathcona chair of pathology.

His friends and colleagues who shared his interest in arteriosclerosis were immensely gratified when the American Society for the Study of Arteriosclerosis created its first annual lecture in the memory of G. Lyman Duff. He enjoyed its meetings, and it is right that his memory should live among them.

G. C. McMillan, M.D.

News and Comment

GENERAL NEWS

Henry Joachim Lecture.—The 19th Henry Joachim Lecture was given at the Jewish Chronic Disease Hospital, Brooklyn, on March 27, by Dr. W. Stanley Hartroft, of the Department of Pathology, Washington University School of Medicine, St. Louis. Dr. Hartroft discussed "Fatty Livers and Sequelae in Experimental Animals and Man: Relation to Obesity, Alcohol and Dietary Protein."

PERSONAL

Dr. W. Stanley Hartroft and Dr. Russell L. Holman at Symposium on Fat in Human Nutrition.—Dr. W. Stanley Hartroft, of St. Louis, and Dr. Russell L. Holman, of New Orleans, participated in a symposium on Fat in Human Nutrition which was held at the Louisiana State University School of Medicine, New Orleans, on March 15. This symposium was sponsored by the Council on Foods and Nutrition of the American Medical Association.

Appointment for Dr. Hans Popper.—Dr. Hans Popper has become full-time director of the Department of Pathology at the Mount Sinai Hospital, New York. He is also Professor of Pathology in the Columbia University College of Physicians and Surgeons.

SOCIETY NEWS

Wisconsin Society of Pathologists.—The Wisconsin Society of Pathologists met in conjunction with the State Medical Society of Wisconsin on May 7 and 8, in Milwaukee. Dr. Jesse E. Edwards, of the Mayo Clinic, addressed both the general session and the pathologists, discussing correlations in "Occlusive Coronary Arterial Disease" and "Surgical Pathology of the Heart."

ANNOUNCEMENTS

Discontinue Shipment of Medical Books to Korea.—The American-Korean Foundation and the United States Army Medical Service have announced the discontinuation of their joint project of shipping medical books contributed by individual physicians, medical schools, hospitals, and state and local medical societies to Korea.

Books should not be sent to the Sharpe General Depot in California as in the past, for facilities no longer exist for packing and transshipping to Korea.

In making the announcement, Howard A. Rusk, M.D., President, American-Korean Foundation said, "The response of physicians and medical groups throughout the country for our appeal for books for Korean medical schools has been so generous that further contributions are not needed." As a result of this program, Dr. Rusk stated, over 77 tons, valued at $76,000, of medical texts, references, and periodicals have been shipped to Korea for distribution to Korean medical schools.

Books

Tumeurs Humaines: Histologie, diagnostics et techniques. Second edition. By P. Masson. Price, paper, 9.4 francs, board, 10 francs. Pp. 1216, with 450 illustrations and 12 color plates. Librairie Maloine, societé anonyme d'éditions médicales et scientifiques, 27, rue de l'ecole de médecine, Paris 6, 1956.

The belated second edition of this long popular standard French work represents the mature opinions of Professor Masson based on a half century of experience. It has been revised and enlarged. The text deals mainly with histology and histogenesis. The illustrations comprise, as before, some figures and photomicrographs but many drawings, effective in their simplicity. A sizable chapter on techniques is included. In his preface the author disclaims any attempt at coverage of the world literature or compilation of all existing knowledge. The book is divided into a general biological part dealing with growth phenomena and a part of eight chapters covering the tumors according to embryological origins of the parent tissues. The amount of space allocated to the individual tumors represents, to some degree, the special interests of the author. The book is a veritable storehouse of information on lesions which superficially resemble neoplasms, entering into differential diagnosis, as well as discussion of the true tumors. The book has the force of fact, which is simply, charmingly, and ably presented.

Histological Appearances of Tumours with a Consideration of Their Histogenesis and Certain Aspects of Their Clinical Features and Behaviour. By R. Winston Evans. Price, $17. Pp. 773, with 989 illustrations. E. & S. Livingstone, Ltd., 16 and 17 Treviot Place, Edinburgh 1, 1956.

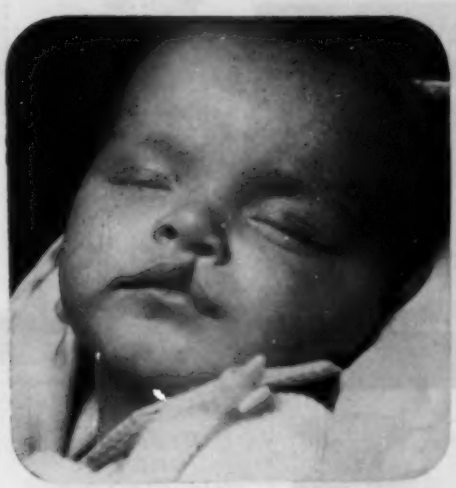
This new book emphasizes the histology and the histogenesis of human tumors. For this purpose illustrations are important. Here they are numerous and well selected; many are superb. Most of the common and many of the uncommon neoplasms are discussed. The author has chosen to omit the tumors of the central nervous system and the female generative organs (except breast), and the coverage of the leukemias is incomplete. Certain proliferative nonneoplastic lesions that enter into differential diagnosis or histogenetical considerations have been included. This category includes among others sclerosing adenosis, cystic mastitis, and the fibromatoses. The references are carefully selected on the whole. In some chapters, as on the thyroid, they are numerous, but in others they are sketchy. Less space is devoted to some of the common tumors (e. g., large intestinal, leukemias, pancreatic) than to others which are far less frequent (e. g., parathyroid, islet cell, thyroid). Although this is satisfactory for the trained pathologist, it is less desirable for the needs of the student in training.

The viewpoints are sound, and the style of presentation is clear and forceful without being dogmatic. This book is different from all others available today. It should be useful in diagnosis to many groups, but especially to the surgical pathologist. It is a welcome addition to the literature on cancer.

Micro-Analysis in Medical Biochemistry. By E. J. King and I. D. P. Wootton. Price, $4. Pp. 292, with 25 figures and 18 tables. Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1956.

This third edition of "Micro-Analysis" was written in conjunction with I. D. P. Wootton, a new co-author with E. J. King. Many new procedures have been added in this edition, and outdated procedures have been eliminated. The book has been limited to routine laboratory techniques in order to keep the reasonably small dimensions of a laboratory manual.

Each technique includes a discussion of principles, procedure, calculation, and reagents used. A list of references permits easy access to additional technical details. Although many of the instruments are British, the procedures can be applied in general to similar instruments available in this country.



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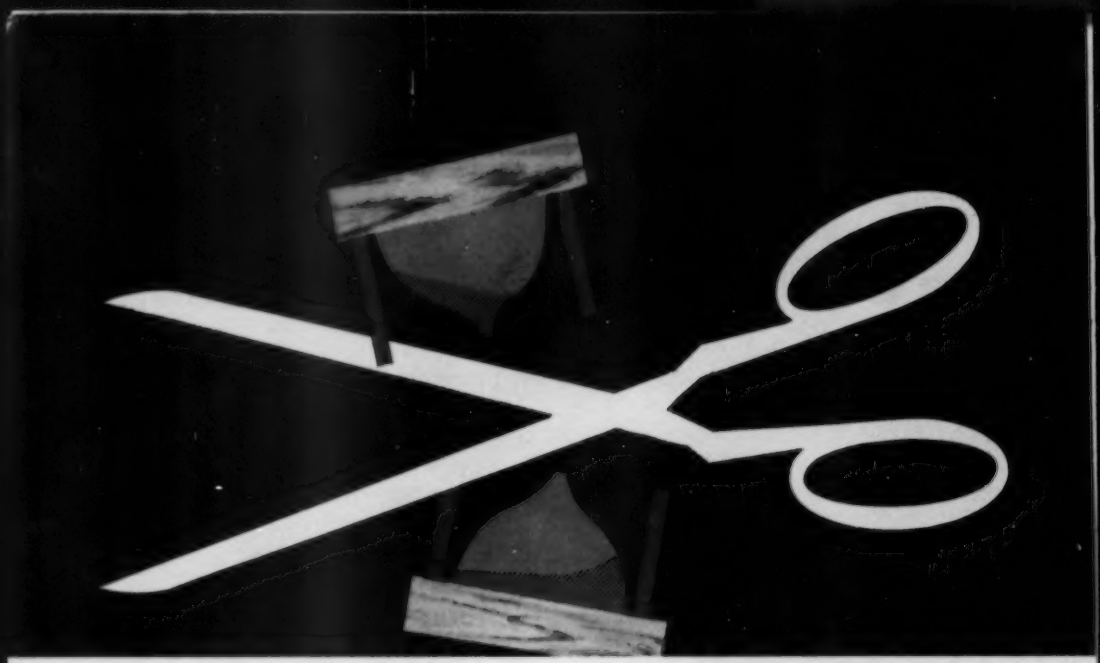

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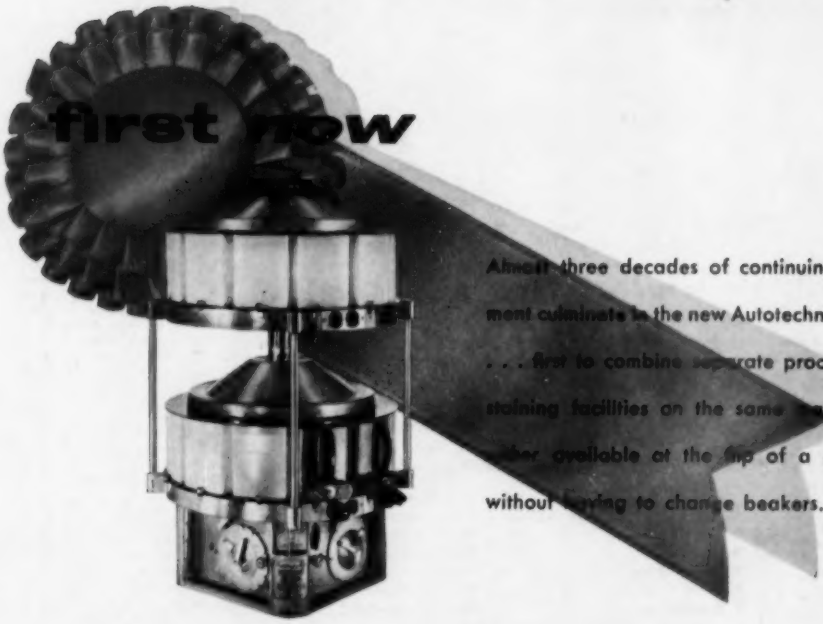

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